EYE PROTECT SYSTEM™ LENSES:

FROM RESEARCH TO HARFMUL LIGHT FILTERING

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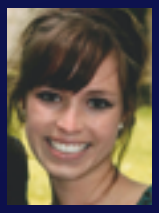
eyeprotect SYSTEM



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Coralie joined Essilor in 2011, after a physics/optics engineering degree from Institut d'Optique Graduate School ParisTech and two Master's degrees with honours from Paris XI in fundamental physics and in optics for new technologies.

Her research is centred on photobiology of the eye, photometry and interferential physics for new ophthalmic healthcare lenses.

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Amelie joined Essilor in 2004 after a Master's degree in chemistry from the graduate school of Chemistry in Montpellier.

Her mission in Essilor is to manage the R&D programs portfolio for the health strategical axis and premium core business: transversal interaction between R&D experts, marketing, engineering and quality, to build programs matching product requests and overall R&D innovation strategy.

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Melanie joined Essilor in 2008 after a 4 years in automative lighting. She holds a physics/optics engineering degree from Institut d'Optique Graduate School ParisTech and a Master's degree in Optics from the University of Arizona (US).

She first joined Essilor's Optics Department working on new products' designs and has been part of the Physics-Chemistry Department. Since 2012, she has been working on radiometry and optical simulation. Her research focuses on the interaction between light, opthalmic lenses and the eye.

INTRODUCTION

Light is a driving force of life, from the most basic function of producing cellular energy to permitting highly sophisticated processes in intelligent life forms. Essential to visual functioning, it brings an unexpected dichotomy to the eye, concomitantly conferring both beneficial and harmful light. Irreversible eye damage from noxious light exposure, which is exacerbated in our currently aging population, has become a preoccupying public health issue.

The major source of light is the sun, emitting harmful ultraviolet (UV) and blue-violet light as well as beneficial blue-turquoise light. Added to this, the development of new sources of artificial light is altering our light exposure profile, increasing exposure to harmful light, with eyes increasingly subjected to potential risks of cumulative retinal damage.

Studying light-induced eye damage is invaluable for designing effective light filtering solutions as part of the preventive tools armamentarium. One of the challenges facing the ophthalmic optics industry is to find the balance between protecting our eyes from noxious light while simultaneously allowing essential light to reach the retina, for both visual and non-visual functions. A better understanding of the biology behind retinal damage is essential for developing refined solutions for adequately protecting our eyes.

In this White Paper we review the current state of research and development, focusing on the role of oxidative stress in retinal phototoaging. We present the new lens solutions put forward by Essilor through their collaborative research with the Paris Vision Institute.

KEYWORDS

harmful blue-violet light, sunlight, light emitting diodes, oxidative stress, ROS, retinal damage, retinal pigment epithelium, phototoxicity, UV, E-SPF®, prevention, Eye Protect System™, Smart Blue Filter™

LIGHT AND THE VISUAL CYCLE

The electromagnetic spectrum and light transmission to the eye

The electromagnetic spectrum covers a continuum of electromagnetic waves, from radio waves, microwaves, infrared, visible and UV radiations, through to X-rays and gamma-rays, the photon energy increasing with decreasing wavelength **[Figure 1]**. Sunlight is composed of 5-10% UV radiation (100-380nm), ~40% visible radiation (380-780nm), and 50-55% infrared radiation. These are either absorbed or transmitted by the successive layers of the eye, modulating the light reaching the retina[1].

UV waves are harmful to the anterior part of the human eye. In a healthy adult's eye no UV radiations actually reach the retina. UVC (100-280nm) from sunlight are filtered by the atmosphere, while most UVB (280-315nm) are absorbed by the cornea. Residual UVB and most UVA (315-380nm) are then absorbed by the crystalline lens. In contrast, visible light reaches the retina in high proportions[2].

In addition to allowing us to perceive the world around us in terms of shape, contrast and colour, visible light also plays an important role in various non-visual functions of the body, controlling many rhythmic biological functions. High energy visible light (380-500nm), commonly known as blue light, accounts for ~25 to 30% of the sunlight within the visible range. It includes both harmful blue-violet radiations (415-455nm) which can be damaging to the retina, but also beneficial blue-turquoise radiations (465-495nm), essential for normal physiological functioning during the day. Although transmission of blue light to the retina decreases with age, as a protection, it nonetheless remains present at significant levels.

Fundamentals of the retinal visual cycle

To reach the retina, light passes first through the cornea, the aqueous humour, the crystalline lens and then the vitreous humour. From here, it crosses the retinal ganglion cells and then several cell layers before reaching the outer retina. The outer retina is composed of retinal pigment epithelium (RPE) cells plus the outer segments of the visual photoreceptors (rods and cones) **[Figure 2]**. The discs of the photoreceptor outer segments (POS) contain visual pigments formed by covalent binding between 11-cis-retinal (a photosensitive derivative of vitamin A) and a transmembrane opsin signalling protein.

Absorbed photons transmit energy to the photoreceptors via the opsin, triggering isomerisation of the 11-cis-retinal which causes a conformational change to all-trans-retinal **[Figure 3]**. The all-trans-retinal is released from the activated opsin into the cytoplasm

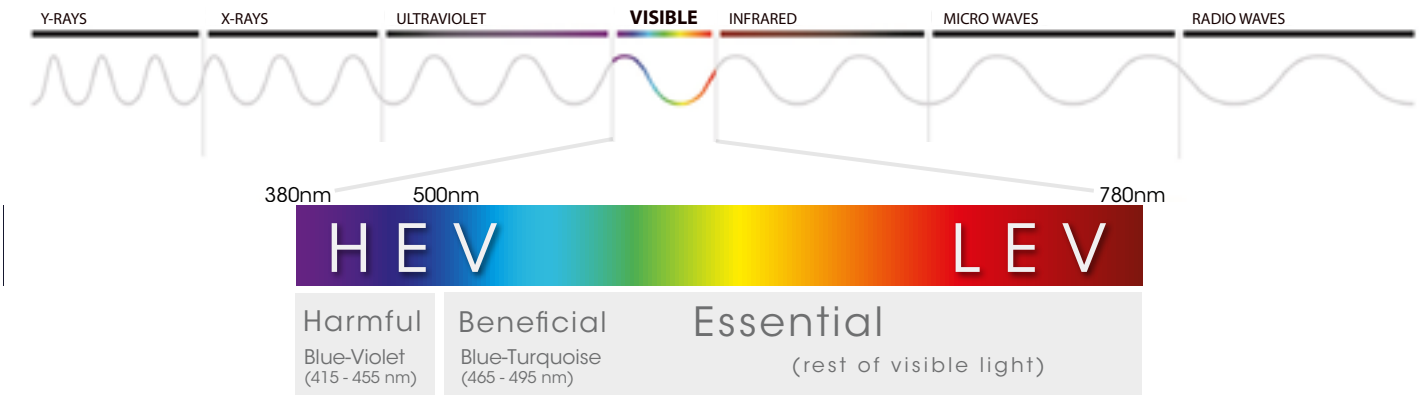

Figure 1. Visible light (380 -780 nm) in the electromagnetic spectrum. HEV-high energy visible; LEV-low energy visible

and is then rapidly reduced to its non-oxidised form all-trans-retinol[3-5], in a healthy retina. This crosses the sub-retinal space and enters the RPE where it is converted back to 11-cis-retinal which returns back to the photoreceptors, binding with opsin, and completing the visual cycle **[Figure 3]**. The RPE plays a critical role in vision; in addition to the constant renewal of 11-*cis*-retinal, it is also responsible for the phagocytosis of the POS discs and providing nutriments and oxygen to the photoreceptors. The visual cycle is the fundamental basis of our vision, and its dysfunction triggers irreversible retinal damage.

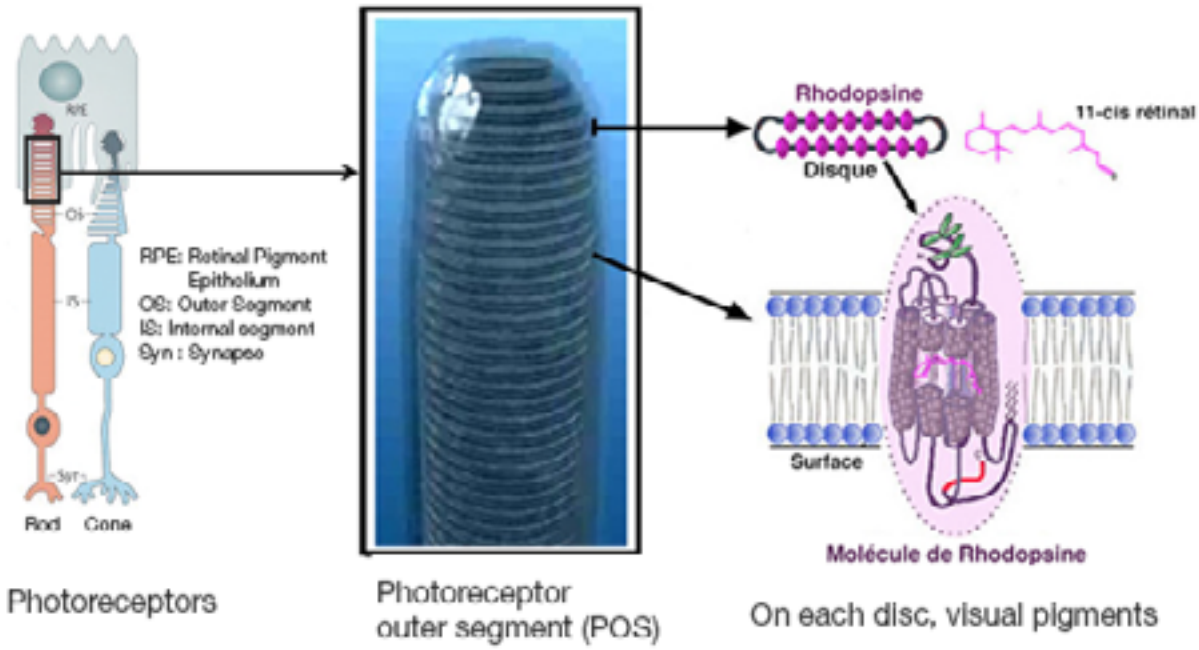

Figure 2. Visual pigments in photoreceptor outer segments

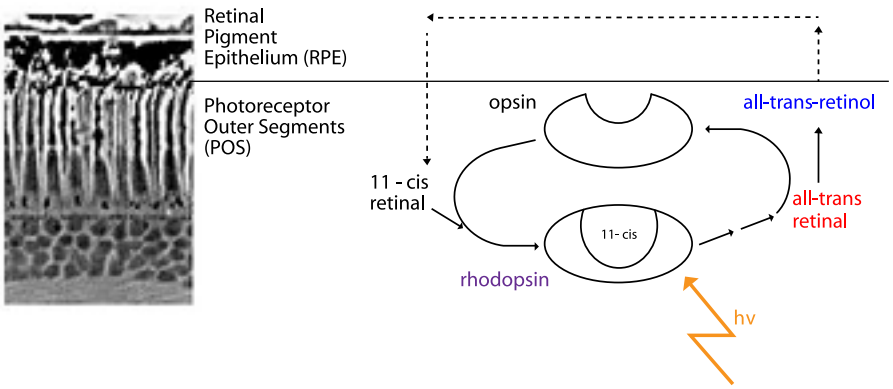

Figure 3. The visual cycle in rods

Eye damage and focus on retinal pathologies

Chronic eye exposure to solar UV waves is associated with the pathogenesis of numerous diseases of the anterior part of the eye, such as pterygium and pinguecula. It is also associated with crystalline lens pathologies, in particular the development of cataracts.

While the visual cycle can be progressively disrupted with ageing, this process is known to be accelerated by light. Retinal damage can originate from photomechanical, photothermal or photochemical reactions. Optical radiation (UV, visible and infrared) has the potential to cause photomechanical and photothermal damage from brief and extreme exposure, while photochemical damage is more commonly due to cumulative and prolonged exposure and is also wavelength-dependent being blue-violet light specific for the outer retina. The cumulative harmful effect of light on the retina depends on the irradiance it receives (i.e. the power received on a given surface per unit area). Retinal irradiance is in turn dependent not only on the light source radiance (i.e. the power of the light source per unit area per unit angle), but also on pupil size (decreases with age and brighter light) and anterior ocular media transmittance.

Among known retinal pathologies, the most preoccupying is age-related macular degeneration (AMD). Along with age, genetics, smoking and diet, blue-violet light is known to contribute to accelerated ageing of the outer retina and is thus a risk factor for AMD[6–13]. AMD involves the degeneration of RPE cells and then the photoreceptors, and is associated with chronic inflammation and oxidative stress. In developed countries, it is the leading cause of irreversible visual impairment, with 17,8 million cases in the US[14] and estimated as 265 million worldwide over the next 30 years. Prevention of retinal damage caused by blue-violet light via photoprotection is an important aspect of optimising retinal health management.

The changing profile of light exposure

Light exposure profiles vary considerably among individuals, integrating a multitude of factors; the type and number of light sources, their localisation, spatial distribution, as well as radiance, spectra exposure duration and repetitions.

Exposure to UV and blue light from the sunlight varies depending on the time of day, geographic location, season, etc., but is also affected by social influences (skin cancer awareness, sunglasses' quality, and social norms relating to skin tanning).

Artificial light sources also contribute to retinal light exposure, altering the light exposure profile with more light sources, longer and repetitive exposure, higher radiance and energy, and at shorter distances. Exposure is occurring in people of all ages, and at increasingly younger ages. Solid-state lighting (SSL) now dominates domestic lighting, with incandescent bulbs being phased out this year, and the European Lighting Industry estimating that over 70% of light sources will be based on SSL by 2020. Current «cold-white» light emitting diodes (LEDs) include up to 35% of blue light within the visible range, compared to incandescent lamps which have less than 5%[15]. «Warm-white» light has less than 10% of blue light but also has lower luminous efficacy. Thanks to their compact form and wide spectral range, LEDs are now extensively used in everyday self-illuminating applications including mobile phones, tablets, computers, TVs and even in toys and clothes. Radiance from LEDs can be up to 1000 times higher than that of traditional incandescent lamps. Combined with the fact that the chronic toxic effect of a light source depends strongly on exposure duration and repetition, this could make LEDs a potential contributor to long-term retinal damage.[16-18]

BACKGROUND RESEARCH ON RETINAL PHOTODAMAGE

Impact of UV on the anterior part of the eye

In the healthy adult's eye, UV radiations are almost totally filtered out by the cornea and the crystalline lens and do not reach the retina. *In vitro*, *in vivo*, and epidemiological data demonstrate that chronic eye exposure to UV radiation is associated with the pathogenesis of numerous corneal and crystalline lens pathologies. The role of UV in corneal damage was shown as early as the mid-1950's when Kerkenezov reported its involvement in the development of pterygium[19]. Since then, numerous *in vivo* and *in vitro* studies using corneas and crystalline lenses from several species (including humans) have demonstrated the higher the wavelength, the higher the UV light damage threshold and thus the lower the toxic effect[20-24].

Weighting the UV hazard spectrum by the sunlight spectral distribution, the greatest danger of UV is in between UVA and UVB with a maximum at around 315 nm.

The mechanisms behind blue-violet light retinal damage

Photochemical damage is mainly associated with long-term and repetitive exposure to moderate irradiances, arising when a photosensitive molecule or chromophore undergoes physico-chemical changes after photon absorption. Damage is dependent on the balance between light exposure and the body's retinal repair systems which manage oxidative stress. These systems are affected by age, genetic and/or environmental factors that can decrease their efficiency.

In the presence of oxygen, high-energy photons can react with photosensitive compounds to produce photochemical reactions and then reactive oxygen species (ROS) including singlet oxygen (O_2), superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO^{-}). These ROS are highly toxic and can cause protein oxidation, lipid peroxidation, mutagenesis, etc[25]. They are naturally derived from numerous intracellular sources including the mitochondria, enzymatic systems or photosensitizers and can occur as a result of exogenous influences such as light, smoking or diet poor in antioxidants.

As one of the highest oxygen-consuming structures in the body[26], the retina is extremely susceptible to oxidative stress. Combined with an abundance of photosensitizers in the outer retina, prolonged visible light exposure and a high energy demand, this gives fertile ground for oxidative stress. The two major photosensitizers in the retina are 11-cis-retinal in the outer segments of the photoreceptors and lipofuscin, a "wear and tear" pigment which accumulates with age in RPE cells[25]. Other photosensitive molecules which may also play a role include cytochrome C, flavins and flavoproteins.

Three major natural antioxidant systems supporting retinal health are superoxide dismutase (SOD), catalase and glutathione **[Figure 4]**. SOD alternately catalyses the partitioning of the ($O_2^{-\cdot}$) radical into "safe" (O_2) or (H_2O_2). (H_2O_2), which is also dangerous, is in turn converted into water (H_2O) and (O_2) by the catalase enzyme or by the glutathione peroxydase enzyme which also converts reduced glutathione (GSH) into oxidized glutathione (GSSG).

When exposed to blue-violet light, all-trans-retinal (which accumulates in the POS), is highly photoreactive and induces oxidative stress, with decreasing sensitivity between 400 and 450 nm. In the absence of sufficient antioxidant activity, the POS progressively oxidises and their renewal within RPE becomes more challenging, generating an accumulation of residual lipofuscin in the RPE[10]. Lipofuscin contains a photosensitizer with a maximum absorption in the blue-violet spectral range at 440 nm. Accumulation of lipofuscin in the RPE is a key feature of ageing and AMD[28,31]. The RPE cells become progressively clogged with age-related waste products, ultimately resulting in apoptosis. Deprived of their support cells, the photoreceptors deteriorate, leading to permanent retinal damage.

Literature review on retinal blue-violet light damage

Retinal damage by blue light has been studied for a

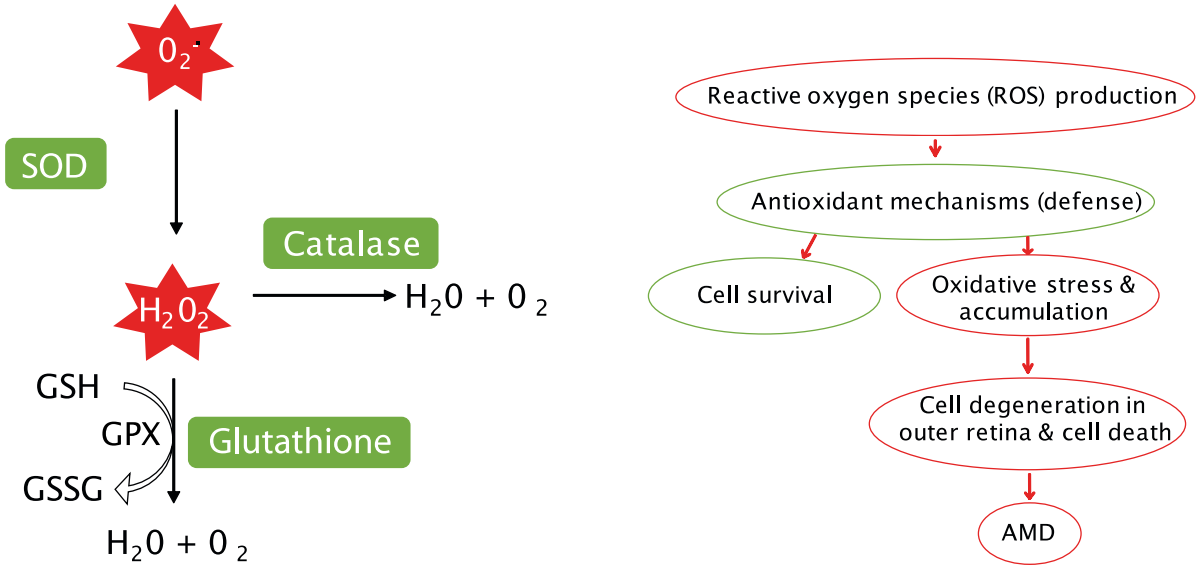

Figure 4. Simplified normal processing of ROS. ROS in red, antioxidant defenses in green (adapted from Jarrett et al., 2012)

half century, starting with the landmark paper published by Noell et al. describing blue retinal phototoxicity in rodents exposed to white fluorescent lamps[30]. *In vitro* studies on immortalised RPE cells loaded with purified lipofuscin showed lower toxicity thresholds with violet-blue-green light (390-550 nm) versus yellow-red light (550-800 nm) [31]. Similarly, human RPE cells loaded with A2E (a well-characterised chromophore in lipofuscin) were approximately 7-fold more sensitive to blue light than to green light[32]. Phototoxicity was not observed without any photosensitizer, and increased with increasing photosensitizer concentrations. This was confirmed in several animal models[33–36]. The role of broadband blue light in oxidative stress was shown in cultured human RPE cells causing lipofuscin-dependent protein oxidation, lipid peroxidation, mitochondrial DNA damage, lysosomal changes and cell death[9,31,37].

Research has been taken a step further with an increasing body of literature studying the impact of LED lighting on the outer retina. A recent *in vitro* study on human RPE cells reported decreased cell viability by up to 99%, increased apoptosis up to 89%, and increased ROS production and DNA damage, after bright exposure to white or blue LED lighting[12]. A similar study on primary human RPE cells reported that cold-white LEDs disrupted the expression of inflammatory markers (VEGF-A, IL-6, IL-8 and MCP-1) and pathological cytokines, and activated relevant signal pathways[38]. A recent *in vivo* study in rats confirmed blue-light dependent damage with a range of coloured LEDs with loss of photoreceptors and activation of apoptosis[39].

Supportive data are found in numerous epidemiological studies suggesting a correlation between blue light exposure from the sun and AMD[40]; in a recent meta-analysis of 14 epidemiology studies, 12 reported an increased risk of AMD with greater sunlight exposure, six of which were significant[13]. Studies of human macular pigment density and the risk of AMD progression following cataract surgery lend further weight to the hypothesis that blue light exposure has a role in AMD pathogenesis, with a three-fold increased risk of AMD progression directly attributed to a dramatic increase in blue light exposure[41–44] after surgery.

in vitro modelling of blue light toxicity on the outer retina (cell death)

While these studies leave little doubt that the outer retina sustains photochemical injury from blue light mediated by the visual pigment for the photoreceptor outer segments and by lipofuscin in the RPE cells, many of the published *in vitro* studies in this field suffer limitations. These include a lack of precision in terms of the light dose and/or use of very high irradiances that can trigger acute light-toxicity mechanisms rather than reflecting lifelong cumulative exposure damage which is more accurately represented by moderate irradiances and longer exposure, particularly in the context of AMD.

In 2011, a fruitful collaboration was developed between researchers at the Paris Vision Institute and at Essilor to address these issues. A well-established *in vitro* AMD model and innovative cell illumination protocol and device were used to evaluate the precise phototoxicity action spectrum (cell apoptosis) occurring under conditions mimicking physiological retinal exposure to sunlight.

Primary swine RPE cells were cultured in the absence of any photosensitizer, then photosensitised with A2E and finally exposed to 10 nm-wide illumination bands across the blue-green range (from 390 to 520 nm in 10 nm increments) plus an additional band centred at 630 nm for 18 hours, using an innovative LED-based fibered device. After light exposure, cells were maintained in darkness for 6 hours then analysed. Moderate irradiances (< 1.6 mW/cm^2 for 630 nm and < 1.3 mW/cm^2 below 460 nm) normalised to the daylight spectrum reaching the retina after being filtered by the ocular media were used. Cell necrosis (reflecting acute light toxicity) and apoptosis (reflecting long-term cumulative light toxicity) were measured[44].

What they found was that firstly, none of the light exposures evaluated altered the necrosis rate compared to cells maintained in darkness, supporting that moderate light irradiance is not associated with acute toxicity. Secondly, decreased cell viability was detected with very low A2E concentrations at 420, 430 and 440 nm, corresponding to blue-violet light. Finally, apoptosis was significantly induced between 415-455 nm **[Figure 5]**, and increased with increasing A2E concentrations. These findings delivered a very precise definition of action spectrum.[45]

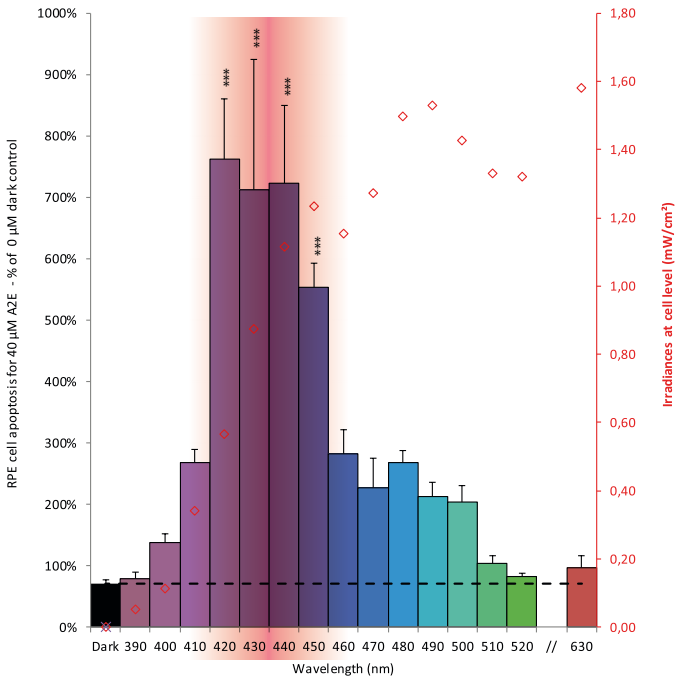

Figure 5. Light toxicity spectrum (apoptosis) in 20 µM A2E-loaded RPE cells after 18 h light exposure.
The lowest the p-value, the highest the significance.
(0.01<=p<=0.05) = *
(0.001<=p<0.01) = **
(0.0001<=p<0.001) = ***
(p<0.0001) = ****
p-value as compared to control cells maintained in darkness.

PAVING THE PATH FORWARD FOR PHOTOPROTECTION

Investigating oxidative stress

Working from the premise that blue-violet light induces apoptotic death in RPE cells, Essilor and the Paris Vision Institute took a step towards fine tuning the understanding of the link between blue light and AMD via accumulation of oxidative stress and modulation of cell defence mechanisms. To identify the role of blue-violet light in accelerating ageing of the outer retina, and more specifically to understand the early stages in this process, they used their cell and light models[45] to address two key questions; does blue-violet light induce ROS and does it inhibit antioxidant defence mechanisms? ..... or both?

As previously described, A2E-loaded RPE cells were exposed to 10 nm-wide light bands within the blue range (390-520nm) and also at 630 nm with a normalised retinal sunlight spectrum. A shorter exposure time was used (15 hours) given that oxidative stress is related to earlier and more sensitive biomarkers. Well-characterised biomarkers implicated in oxidative stress were analysed. Two main ROS, (H_2O_2) and ($O_2^{-\cdot}$), were used as a measure of change in the balance between ROS generation from light exposure according to their elimination by antioxidant mechanisms **[Figure 3]**. Blue-violet light exposure is associated with high levels of (H_2O_2) and ($O_2^{-\cdot}$), significantly higher than control levels (in darkness) with the greatest effect observed between 415 nm and 455 nm **[Figure 6]**, corresponding exactly to the toxic spectral band associated with cell apoptosis[45] **[Figure 5]**.

To answer the complementary question as to the effect on oxidative stress repair processes, researchers used a three-pronged approach, evaluating the effects of blue-violet light on glutathione conversion, as well as SOD and catalase activity and also mRNA expression levels of SOD 2 (mitochondrial SOD), catalase and glutathione peroxydase (GPX1) (enzyme that catalyzes the conversion of GSH to GSSG).

GSSG concentrations, the oxidised form of GSH and as such an indicator of oxidative stress, increased following blue light exposure at 400, 440 and 480 nm, suggesting that glutathione antioxidant mechanism tries in the first place to compensate the high quantity of (H_2O_2) in these light conditions, especially for blue-violet light **[Figure 7]**. In addition, mRNA expression levels of the enzyme glutathione peroxydase (GPX1) decreased significantly, showing that even though glutathione is more active just after blue light exposure, the defence mechanism will be progressively disrupted (a drop in mRNA expression induces a drop in protein synthesis and thus a decrease of

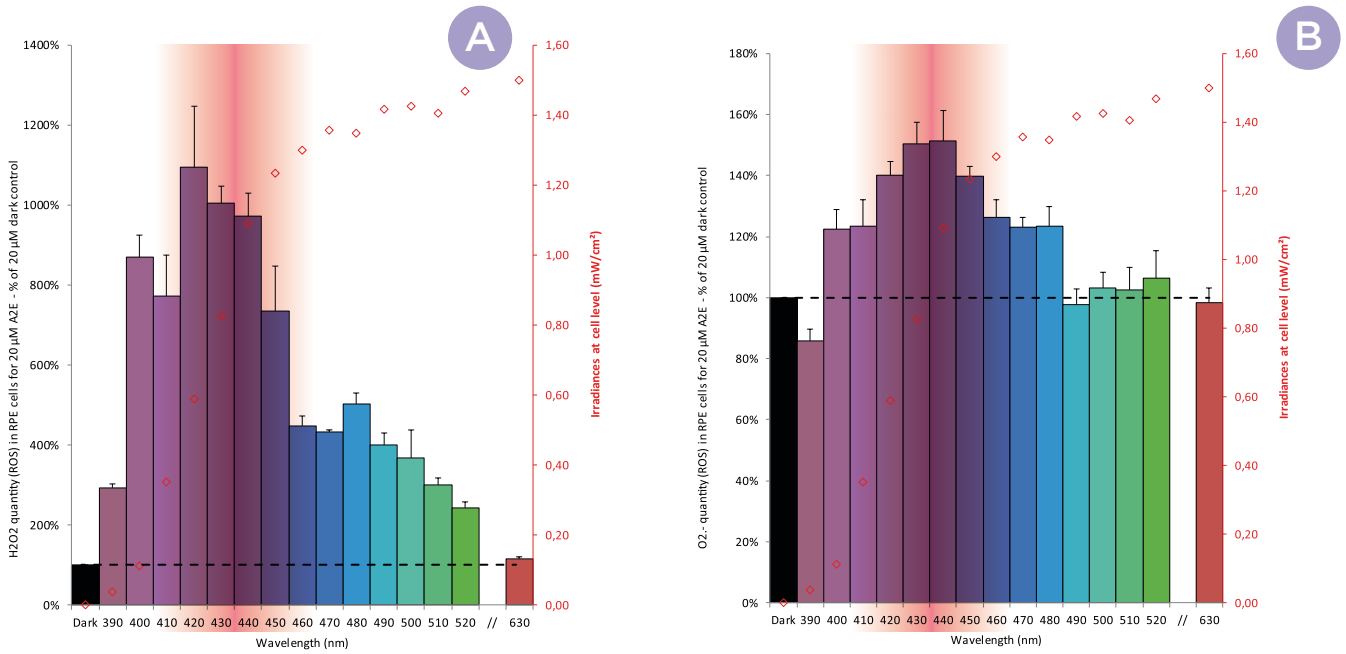

Figure 6. Blue-violet light is associated with significant increases in ROS levels in 20 µM A2E-loaded RPE cells after 15h light exposure for H_2O_2 (A) and $O_2^{-\cdot}$ (B);
Do not disclose, do not copy without written authorization. Paris Vision Institute & Essilor R&D results. Submission of scientific paper in progress. Results presented in ARVO 2015 (poster + short lecture)

glutathione peroxydase production, preventing from GSH and (H_2O_2) to be converted to GSSG, (H_2O) and (O_2)).

SOD activity was also significantly increased by blue-violet light at 440 nm suggesting SOD is first strongly activated in an attempt to compensate the increased (O_2^{-}) production. SOD2 mRNA levels were significantly decreased, highly suggesting the defense enzyme tends to be disrupted and decreased, and thus less functional **[Figure 8]**. There was also an almost complete reduction in catalase activity with blue-violet light at 430 and 440 nm (with or without A2E), suggesting a reduced antioxidant capacity.

The significant increase of GSSG quantity and in SOD activity just after exposure to 400-480 nm wavelengths reflect the cell's attempts to rapidly defend itself against the increased ROS production, however this increased activity failed to compensate the increased (H_2O_2) and (O_2^{-}) accumulation only in the blue-violet range (more in between 415-455nm).

The significant reduction of mRNA expression levels of SOD 2, GPX1 (glutathione peroxydase) and cata-

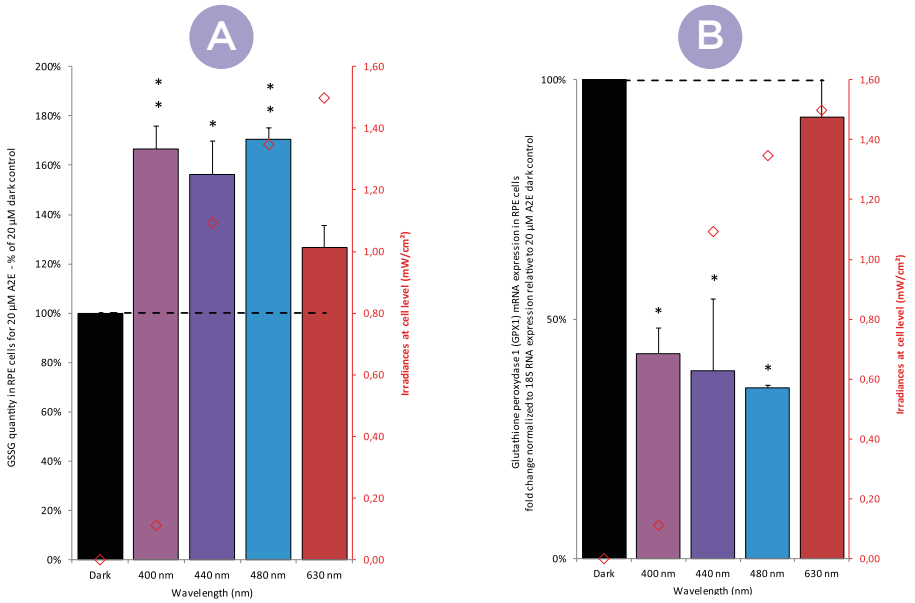

Figure 7. GSSG concentrations (A) and GPX1 mRNA expression levels (B) in 20 µM A2E-loaded RPE cells after 15h light exposure.

The lowest the p-value, the highest the significance. (0.01<=p<=0.05) = * (0.001<=p<0.01) = ** (0.0001<=p<0.001) = *** (p<0.0001) = **** p-value as compared to control cells maintained in darkness.

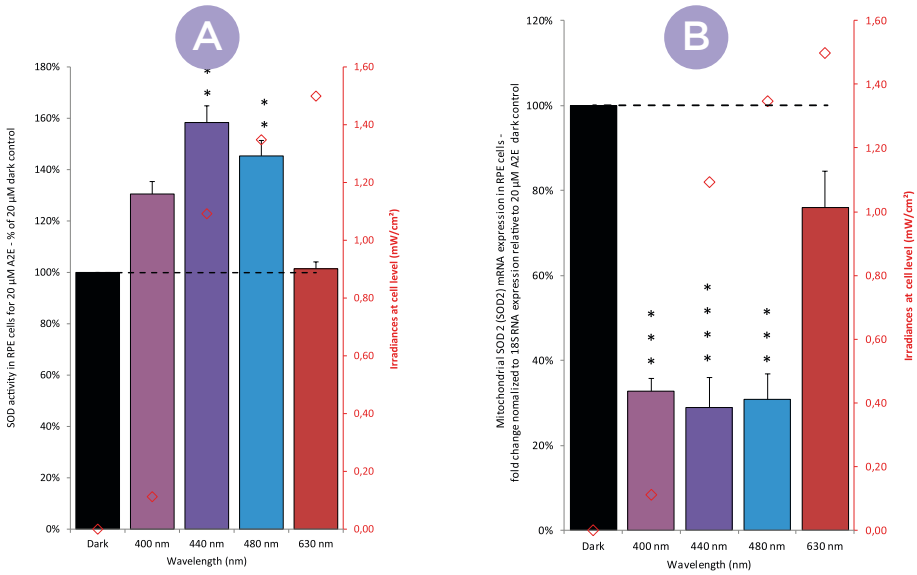

Figure 8. SOD activity (A) and SOD 2 mRNA expression levels (B) in 20 µM A2E-loaded RPE cells after 15h light exposure.

The lowest the p-value, the highest the significance. (0.01<=p<=0.05) = * (0.001<=p<0.01) = ** (0.0001<=p<0.001) = *** (p<0.0001) = **** p-value as compared to control cells maintained in darkness.

lase in the blue range supports the hypothesis of a progressive strong decrease in protein synthesis and thus, at the end, a strong decrease of the 3 antioxidant mechanisms.

Finally, further proof of oxidative stress with blue-violet light exposure (at 420, 430, and 440 nm) was obtained in terms of its effects on the mitochondria, with a modified cellular distribution restricted within the perinuclear area **[Figure 9]**, along with an altered morphology (from tubular to globular). A statistical drop in the mitochondrial respiration rate impacting ATP production was also seen at 440 nm (compared to 400 and 480 nm), reflecting a major metabolic defect under blue-violet light exposure.

Researchers thus confirmed that blue-violet light results in increased ROS production in RPE cells. Also, blue-violet light decreases cell's self-defence systems, making them inadequate to compensate ROS increase.

A need for blue-violet light protection

With clear experimental data confirming that blue-violet light acts as a strong inducer of oxidative stress on the outer retina, there is a growing need for blue-violet light protection against retinal pathologies, and in particular AMD. Photobiology safety standards for lighting products need to keep up with our rapidly evolving light exposure profile, maintaining a strong focus on conducting health risk assessments and extensive experimental studies on LED-based systems. Blue light protection norms are trailing behind those of UV light, with no current standards for cumulative toxicity limits.

Various international initiatives have highlighted concerns over potential health issues of solid-state lighting, including the ANSES 2008 task group (the French Agency for Food, Environmental and Occupational Health & Safety) and the 2014 SSL Annex (4E IEA), calling for photobiological safety assessments for all SSL devices (LED-based) using the joint CIE S009 / IEC 62471 standard. Risky light exposure profiles need to be identified and related to high-risk populations (pre-existing eye conditions, children and the elderly, etc). A new ANSES task group was formed in 2015 to address these issues head-on.

It is important to keep in mind that blue light encompasses wavelengths which perform essential functions. These key metabolic non-visual functions driven by blue-turquoise light including circadian resetting, melatonin suppression, pupil light-induced reflex, cognitive performance, mood, locomotor activity, memory, body temperature, etc; are thought to be mediated by photosensitive retinal ganglion cells containing the melanopsin photopigment with an absorption peak at 480 nm. When designing ophthalmic lenses, it is thus essential to filter only the harmful blue-violet light while ensuring beneficial blue-turquoise light reaches the retina during the day.

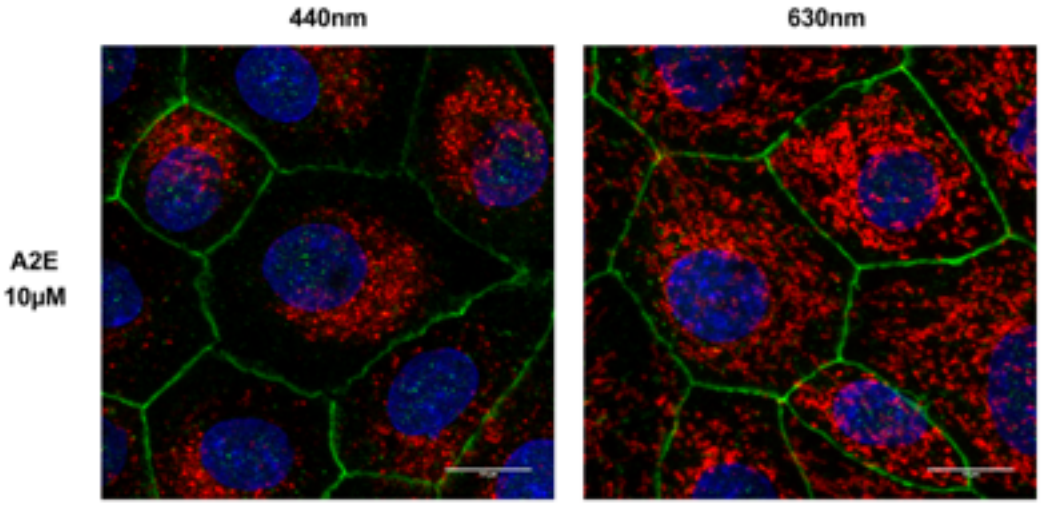

Figure 9. Altered mitochondrial cellular distribution after 15h light exposure at 440 nm in 10 µM A2E-loaded RPE cells (left) compared to 630nm (right)

HIGHLIGHTS ON RESEARCH WORK

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For over half a century, a large body of *in vitro* and *in vivo* experimental evidence has progressively revealed a strong scientific rationale for blue-light induced toxicity in the outer retina. Many of these studies suffer limitations such as not evaluating the toxic risk of each blue wavelength or illuminating with very high irradiances that trigger acute light-toxicity mechanisms rather than cumulative exposure damage which should be sought when studying the pathogenic mechanisms of AMD. To go a step further from a photometry standpoint, we joined skills with the Paris Vision Institute in 2008. In research performed prior to 2013, we scanned the phototoxic risk of each 10 nm band of the blue-green spectral range, simulating physiological retinal exposure to sunlight. Since 2013, we focused our research on the comprehensive understanding of the role of blue light on each step of the RPE cell degenerative process, from the earliest stages through to cell death. We explored the photomodulation of oxidative stress and cell defense mechanisms in the outer retina with two questions in mind. First, does blue-violet light act as an inducer of reactive oxygen species? Second, does blue-violet light act as an inhibitor of antioxidant defense mechanisms?

Very interestingly, in 2013, we found that it is a narrow spectral range, blue-violet light from 415 to 455 nm, that induces the highest apoptosis of RPE cells (Arnault et al., PlosOne, 2013).

In 2015, we further confirmed this specific toxic action spectrum of light with oxidative stress biomarkers. First, we highlighted a strong accumulation of reactive oxygen species in response to blue-violet light. Second, we demonstrated that blue-violet light also acts as a strong inhibitor of antioxidant defense mechanisms. This means that blue-violet light is not only a strong stress inducer but also a defense inhibitor. This double negative effect strongly supports the hypothesis of blue-violet light as an important contributor of oxidative stress in the earliest stages of cell damage, and thus of accelerated retinal ageing, potentially leading to cell death and ultimately to faster AMD onset or progression.

As age-related oxidative changes in the outer retina are a hallmark of early AMD, the identified deleterious effect of blue-violet light at each step of the damaging cycle of RPE cells strengthens the role of blue-violet light as an initiating cause of AMD. Together, our latest photobiology data provide strong scientific evidence on the role of blue-violet light induced retinal damage, providing comprehensive evidence that the most harmful light band to RPE cells is indeed between 415 and 455 nm.

PHOTOPROTECTION: FROM CELL RESEARCH TO LENSES

A timeline of smart filtering protective lens

The last decade has seen major innovations in clear everyday lenses with the incorporation of evolving photoprotective technology. Essilor's photoprotective research program started about 10 years ago, delivering in 2011 the first antireflective coating with low UV reflection on back-side with Crizal® UV.

Thanks to the collaborative research program between the Paris Vision Institute and Essilor, the Crizal® Prevencia® coating was released in 2013, the first clear lens to integrate an antireflective coating that filtrates both UV and partially harmful blue-violet light while maintaining a maximum of the essential blue-turquoise light.

2016 brings Essilor's latest technological advance, the Smart Blue Filter™, a novel approach using embedded blue-violet light protection, compatible with any antireflective coating. The Eye Protect System™ lens brings together the Smart Blue Filter™ and UV protection while ensuring minimal aesthetic compromise. Bringing protection to a higher level, the Smart Blue Filter™ lens has been combined with Crizal® Prevencia® coating.

Refining the test system: a new adjustable fibered white light device

Today Essilor has the edge in the ophthalmic optics industry, by testing *in vitro* the photoprotective potency of lens filters. The photoprotective effect of the Smart Blue Filter™ lens feature was compared between each narrow illumination band within the blue-violet range from 400-450 nm to better differentiate the different spectral profiles.

To validate the photoprotective effect in real light conditions, a polychromatic light source (as opposed to the monochromatic light source used in the *in vitro* model) was envisaged.

Over an 18-month period, researchers at Essilor developed an innovative adjustable fibered white light illumination device which can generate programmable and variable spectra and irradiances within the visible range. This new device offers greater flexibility than the previous blue-green light device, delivering any spectrum within the visible range, thus in addition to daylight spectra it can also mimic warm-white or cold-white LED, fluorescent, and incandescent spectra, and even quasi-monochromatic light. The photoprotective potency of the Smart Blue Filter™ lens feature was measured in terms of the reduction in apoptotic cell death with the filter versus without.

UV protection: the E-SPF® index

UV is a constant source of potential eye damage irrespective of the weather conditions. Exposure occurs directly from the sun's rays, however more than 50% of UV radiation reaching the eye is indirect, coming from cloud scatter and reflection[46]. Public awareness of UV eye hazards has been increasing since the widespread UV SPF campaigns to protect against skin cancer.

Most clear lenses provide a high level of UV protection by absorption. Crizal Forte® UV coating offers additional UV protection on the back surface of the lens to limit UV reflection that can reach the eye.

The E-SPF® (eye-SPF) index takes into account both UV transmission through the lens and UV reflection off the back surface of the coated lens, although it does not account for light coming around the lens or for variations with facial morphology, gaze direction and glasses shape. Essilor is currently offering an E-SPF® up to 35 on clear lenses.

Global protection with the Eye Protect System™ lens

The Smart Blue Filter™ innovation was designed to distinguish harmful blue-violet light from essential blue-turquoise light, absorbing the former and transmitting the latter, using specific absorbers for blue-vio-

let light, embedded inside the lens such that blue-violet light reaching both the front and the back-surface of the lens is filtered **[Figure 10]**.

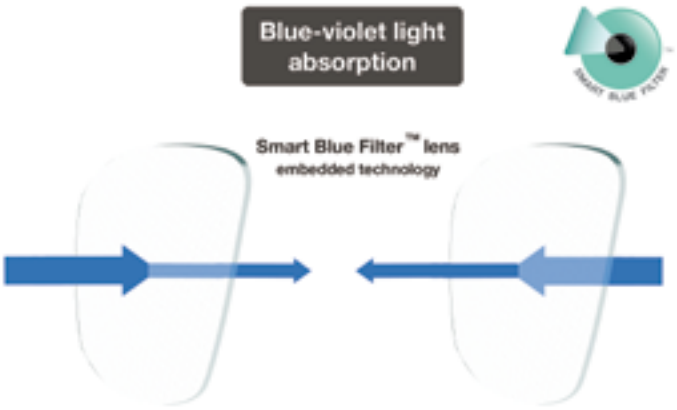


Figure 10. Blue-violet light reaching the lens from the front or back surface is absorbed by the embedded filter, while not affecting other wavelengths

This new embedded innovation offers the key advantage of being compatible with all antireflective coatings. Building on the existing Crizal® UV coating, Essilor combined the embedded Smart Blue Filter™ feature with E-SPF®, offering a clear lens with both UV and blue-violet protection called Eye Protect System™.

As such, the lens filters on average 20% of the blue-violet light between 400-455 nm, combined with UV protection **[Figure 14]**. Its blue light photoprotective effect on retinal cells (RPE) *in vitro* is equivalent to that offered by Crizal® Prevencia® coating giving a 25% (±5%*) decrease in retinal cell death.

A new lens without aesthetic compromise

The absorber filtering partially blue-violet light attributes a natural yellow-orange colour to the lens which is not acceptable for a clear day to day lens. To counteract this, two neutralising molecules were added to the Smart Blue Filter™. To assess the accuracy of transparency, a sensory analysis** with trained judges was performed evaluating four parameters; lens colour

* Standard deviation based on a calculation model for all substrate
** EUROSYN Sensory Analysis (N=13 trained judges, Quantitative Descriptive Analysis) FRANCE – 2015.

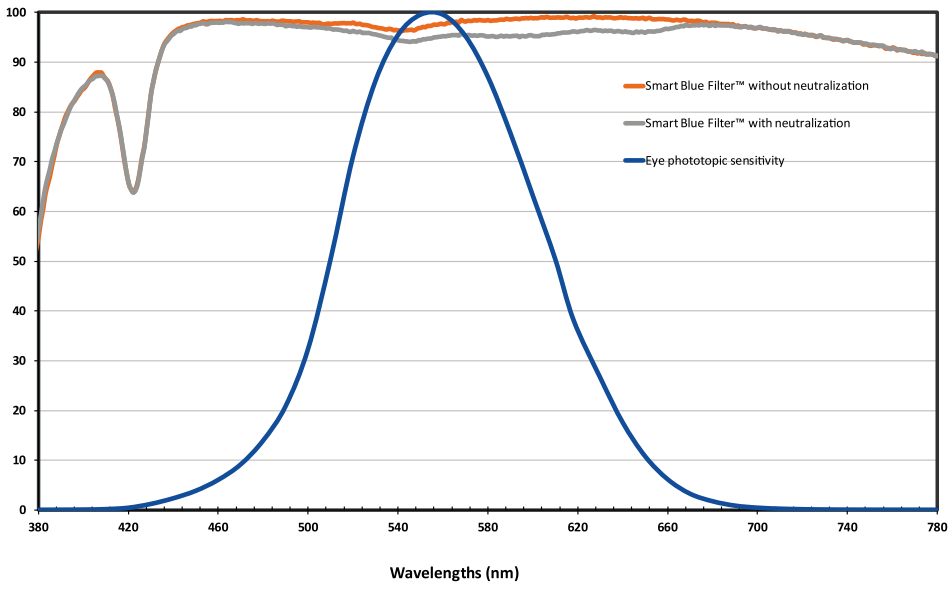


Figure 11. Effect of colour neutralisation on transmittance on clear lens. Both lenses with Crizal Forte® UV coating and transmittance were measured on prototypes 1.5-index CR39 Plano (2 mm centre thickness).

(through the lens for the wearer), skin colour (through the lens for the observer), lens transparency (through the lens for the wearer and observer), and perception of a colour picture (through the lens for the wearer). All parameters consistently rated better for the neutralised lens than for the yellow-orange one.

Transmittance was almost identical between a yellow-orange lens and a neutralised lens **[Figure 11]**. In addition to the sensory analysis, a consumer test* was performed to assess the acceptance of this new lens without explanations on the additional benefit brought. After one month of wear, 96% were satisfyed by its aesthetic. This test shows that the new embedded technology is not noticeable by wearers.

*EUROSYN Acceptance Wearers Test (N=57 lens wearers wearing previously lenses with Crizal® coating that have been equipped with Eye Protect System™ lenses with Crizal Forte® UV coating (same index and Rx) / results after 1month) – FRANCE – 2016.

Essilor Ultimate Protection: the Eye Protect System™ lens with Crizal® Prevencia® coating

Combining the embedded Smart Blue Filter™ feature with the Crizal® Prevencia® antireflective coating gives a maximized protection; blue-violet and UV reflection off the front-surface with Crizal® Prevencia® coating, partial blue-violet and UV filtration by the Eye Protect System™ lens itself, and minimisation of UV reflection off the back-surface of the lens with Crizal® Prevencia® coating **[Figure 12]**. Combining these two solutions of blue-violet filtering offers a maximized protection for a clear lens, filtering on average 30% of blue-violet light (1.59 index lens) between 415 nm and 455 nm and reducing retinal cell apoptosis by 35% (±5%**), approximately 10% more than with Crizal® Prevencia® coating alone.

** Standard deviation based on a calculation model for all substrate

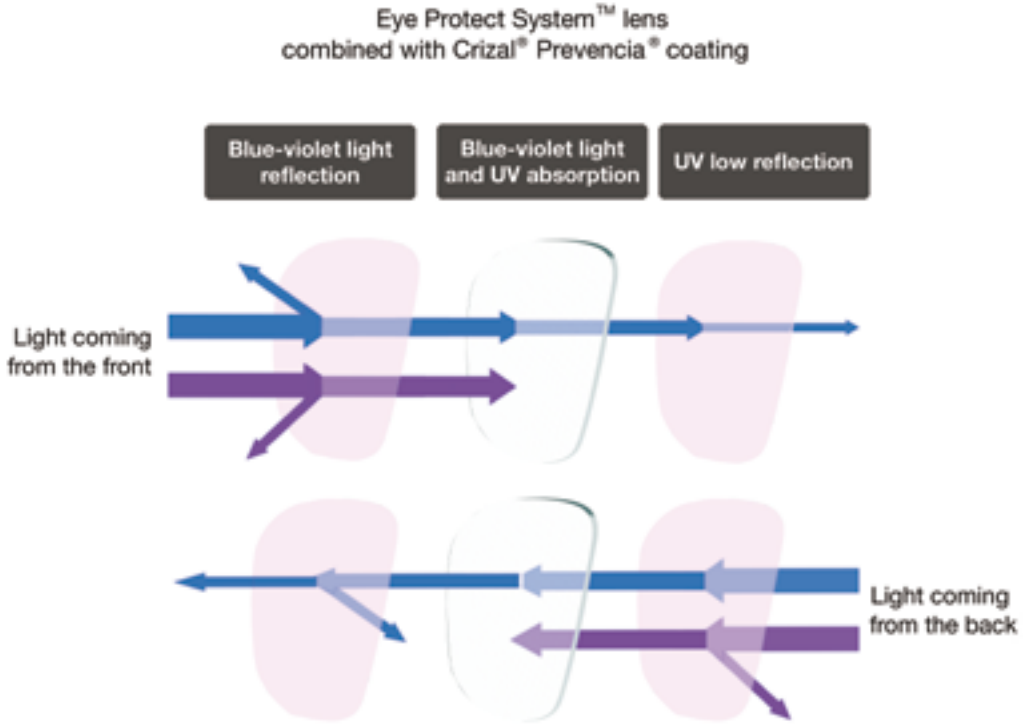


Figure 12 - The Eye Protect System™ lens combined with Crizal® Prevencia®coating ensures filtering of harmful UV and blue-violet light protection while ensuring transmittance of valuable blue light.

The Eye Protect System™ lens with Crizal® Prevencia® coating offers a transmittance profile filtering the greatest proportion of the harmful blue-violet light while allowing the beneficial blue-turquoise light through **[Figure 13]** with comparable lens aesthetics to Crizal® Prevencia® coating alone.

Finally, the Eye Protect System™ lens with Crizal® Prevencia® coating offers a significant reduction in discomfort glare compared to a standard Crizal Forte® UV lens. A study* with nine young healthy subjects suffering moderate or high photosensitivity showed the highest photosensitivity threshold was with the Eye Protect System™ lens with Crizal® Prevencia® coating, giving a 1.5-fold improvement in discomfort and glare compared to Crizal Forte ® UV coating.

* Essilor R&D study (N=9 Discomfort glare & Blue-filtering lenses Focus on Smart Blue Filter™ with Crizal® Prevencia® coating) - FRANCE - 2015

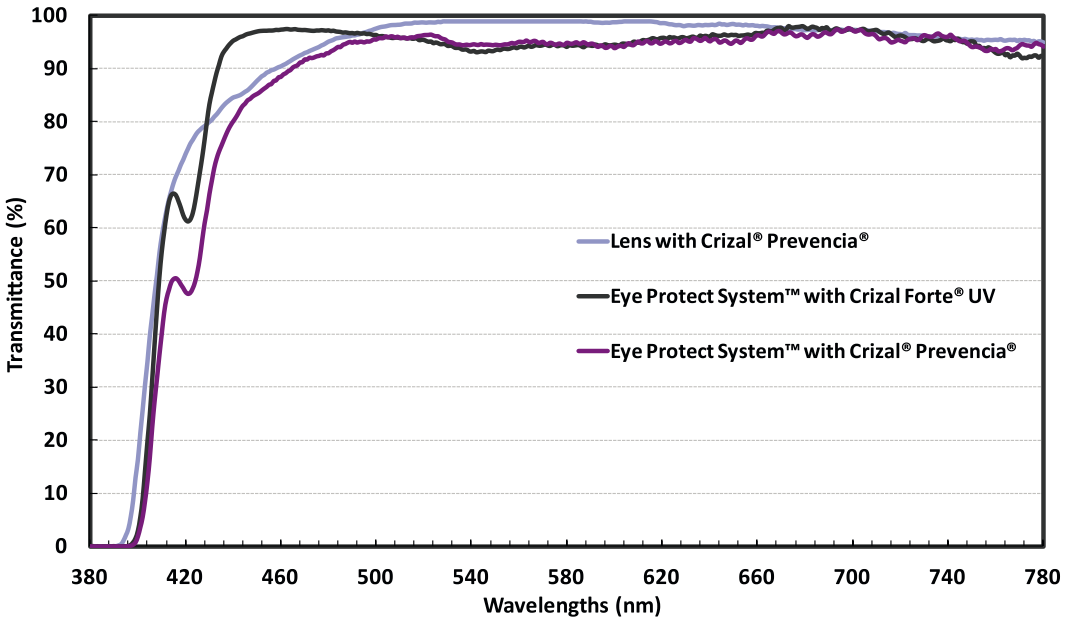
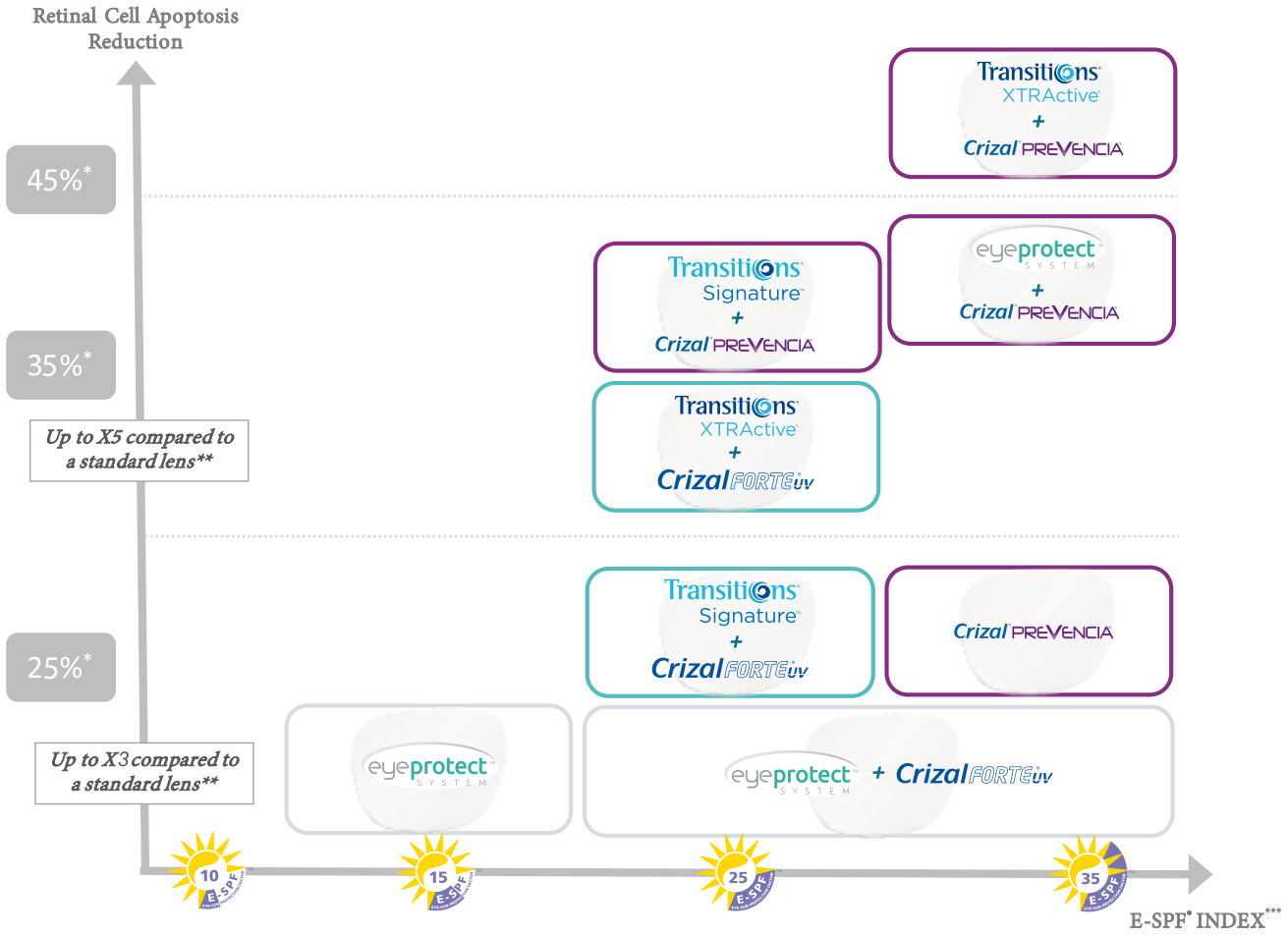


Figure 13 - Transmittance curves of an Eye Protect System ™ clear lens with Crizal® Prevencia®coating

Essilor's current range of Eye Protect System™ lenses*

At Essilor, we recommend three levels of protection; ESSENTIAL composed of the Eye Protect System™ lens (HC version or antireflective with E-SPF® 10), ADVANCED UV protection adding a UV optimized back-side AR coating such as Crizal Forte® UV with E-SPF®25 or E-SPF®35 and ULTIMATE UV protection and partial blue-light filtration with the antireflective coating Crizal® Prevencia® **[Figure 14]**.

* The commercial offer can differ depending on country



** +/-5% standard deviation based on a calculation model for all substrate*
*** Up to 3 times / 5 times more protective against Blue-Violet light than standard prescription lenses, based on in vitro photoprotection tests on retinal cells.*
For Eye Protect System™ lenses with Crizal Forte® UV coating, 25% (+/-5%) decrease in light-induced retinal cell death versus no filter.
For Eye Protect System™ lenses with Crizal® Prevencia® coating, 35% (+/-5%) decrease. For standard lenses: 1.5 or Poly material with Crizal Forte® UV coating, about 7% decrease (mathematically modeled)."
Standard AR lens = a lens with an AR coating, non-UV optimized, without blue protection.
**** The E-SPF® index depends on the lens material itself and the coating. E-SPF® is an index rating the overall UV protection of a lens. E-SPF® was developed by Essilor International and endorsed by 3rd party experts. Lens performance only. The E-SPF® index excludes direct eye exposure that depends on external factors (wearer's morphology, frame shape, position of wear).*

Figure 14 - Range of Eye Protect System ™ lenses

KEY FACTS

PHOTORECEPTION

- Blue light encompasses both harmful blue-violet radiations (415-455 nm) which can damage the retina and beneficial blue-turquoise waves (465-495 nm) essential for normal physiological functioning during the day (rhythmic biological functions).
- The visual cycle, highly involving retinal pigment epithelium (RPE) is fundamental to vision and its progressive dysfunction may be associated with retinal pathologies.
- Blue-turquoise light needs to be transmitted by the lens, especially during the day.

PHOTOTOXICITY & NEW PHOTOBIOLOGY RESEARCH

- UV is a risk factor for diseases of the anterior part of the eye (cataracts...).
- Lipofuscin, the age pigment, accumulates with age in the outer retina, and reacts with energetic blue-violet light, which contributes to accelerated photo-ageing of the outer retina.
- The toxic action spectrum of light on the outer retina (RPE cells) is identified as blue-violet light 415–455 nm (Arnault, Barrau et al., PlosOne, 2013).
- New data confirmed:
  - The toxic action spectrum with oxidative stress biomarkers.
  - Blue-violet light induces high ROS accumulation (H_2O_2, $O_2^{-\cdot}$): it is a STRESS INDUCER
  - Blue-violet light acts as a strong inhibitor of antioxidant mechanisms (glutathione, SOD, catalase): it is a DEFENSE INHIBITOR
  - Blue-violet light directly impacts mitochondria: peri-nuclear clustering, globular shape, decreased respiration rate.
- Low-irradiance blue-violet light induces apoptotic cell death.
- Cumulative (i.e long-term with moderate irradiance) damages induced by light are wavelength-dependent and relevant for eye ageing.
- Blue-violet light is an accelerator of retinal ageing: it is a risk factor for AMD.

PHOTOPROTECTION

- Essilor is the first ophthalmic actor to conduct *in vitro* tests to assess the photo-protective potency of lenses.
- The Eye Protect System™ lens protects against both harmul UV and blue-violet light.
- 3 levels of protection are available with increasing E-SPF® and blue-violet light protection from Essential, Advanced to the Ultimate level which also offers an extra filtering of blue-violet light.

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