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# Extending Human Vision to Infrared and Ultraviolet Light: A Study Using Micro-Particles and Fluorescent Molecules

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**ABSTRACT** In this study, rare earth doped infrared (IR) to visible, up-converting particles along-with efficient ultraviolet (UV) to visible fluorescent molecules were imbedded in proteins and were used as a mean for increasing the human vision range to infrared and ultraviolet wavelengths. Stilbene-420 fluorescent molecules which convert near UV light to blue light, were chosen for the strong overlap of their fluorescence emission with the sensitivity region of blue cone cells and for the ability of blue light to increase the regeneration of bleached visual pigments. Our data show that the up-conversion efficiency of the IR up-converting particles and blue fluorescing molecules efficiencies remained unchanged when these material are imbedded in proteins compared to their efficiencies in water solutions. Addition of up-converting particles to rod visual cells resulted in the bleaching of the visual pigments, rods, when irradiated with infrared light (980 nm) whereas no bleaching was observed, under the same conditions, without the presence of up-converting particles. This suggests that the up-converted green light induced visual process in the rod visual pigments. In addition, we describe the design and present data of a novel optical device, which can be used as eye glasses, utilizing up-converting particles that allows the wearer to see rather intense infrared light.

**INDEX TERMS** Extended spectral vision, green fluorescent protein, rare earth doped upconverting particles.

## I. INTRODUCTION

Human eyes do not operate at all wavelengths. In fact, the sunlight outside the 400-750 nm spectral region is invisible even though a very considerable flux of sunlight reaching the earth's surface is in the UV and IR regions.

The objective of the research presented in this article has been to extend the visual perception region of the human eye by: (a) utilizing up-conversion particles to convert infrared light to visible light which is achieved by multiple sequential excitation of a series of meta-stable excited states to an upper state which emits at shorter wavelengths [1] and (b) conversion of UV light to visible by emission of high efficiency fluorescence by dye molecules, thus extend the visual perception of human and animal vision to the ultraviolet and infrared regions. This visual system is expected to find several applications such as, space, security and possibly tackling eye diseases such as color blindness and many others.

To convert infrared light to visible, probably, the most frequently used up-converting materials are rare earth ions in the $4f^N$ state [2]. Multi-photon, IR to visible up-conversion is achieved in rare earth ions such as $Er^{3+}$, that exhibit equally spaced intermediate excited states, which are pumped by a narrow bandwidth IR laser, to an upper level which subsequently emits in the visible region. To that effect, five photon up-conversion using 1.5 $\mu$m narrow band photon energy and 4-photon up-conversion with 980 nm pumping laser has been achieved in $Er^{3+}$ [1], [3]. The 4f states of these rare earth ions

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are localized, therefore, quantum confinement effects are not expected for the localized electronic states of the rare earth ions doped in insulating nano- or micro-particles. Crystal field theory, suggest that two ion interactions may be treated by an operator Hamiltonion defined, in the crystal field part as:

$$H_{\mathrm{cf}} = \sum\nolimits_{k,q,i} B_q^k C_q^{(k)}(i).$$

The summation for $i$ is over all equivalent open cell electrons, $B_q^k$ are crystal field parameters, $C_q^{(k)}$ are tensor operator components, acting on the free-ion wave-function for $f$ electrons. Here, $k$ takes values 0 to 6 and $q$ from 0 to +/- $k$ which satisfy angular momentum coupling rules and $B_q^k$ refer to the lattice contribution which is dependent upon ligand distance and symmetry [3], [4].

The spectroscopic properties of the doped emitting particles depend on the vibrational modes and local crystal structure. In fact, the lifetime of the excited states, of the doped $Yb^{3+}$ and other rare earth ions, are strongly dependent on the particle size and also on their environment. The crystal structure of the upconverting particles also has a strong effect on the efficiency of upconversion. It has been shown that hexagonal $\beta NaYF_4$ activated by $Yb^{3+}$, $Er^{3+}$ is an order of magnitude more efficient than $\alpha NaYF_4$ crystal [3], [5].

It has also been shown that the size of the crystal may change significantly the lifetime and the particle sizes also have large effect on the intensity and charge-transfer energy transition [6]. To that effect, it has been shown [7], [8] that one may change the emitted color of rare earth doped nano-crystals by modifying the size of the crystals. This change in emission color of the $Er^{3+}$ doped crystals is not due to spectral shifts, but rather due to change in relative intensities of green, red and blue up-conversion which are a function of the host particle size and doping concentration, pumping power and temperature, which are attributed to the excited state phonon assisted radiation-less decay and energy transfer [1], [9].

Another very important aspect is that up-conversion is highly dependent upon the strength of the vibronic side bands. To that effect, it is well known that vibronic transitions of the $4f^N$ states play a very important role and highly influence up-conversion.

However, the increase of non-radiative transitions are reflected in an increase in the excited state(s) decay rate. While, the electronic transition between two states, a and b with energy separation $E_{\mathrm{ab}}$which is coupled to a vibration mode $\nu$ is:

$$f\,(E) = \exp(-S)\frac{S^n}{n!}\frac{1}{\sqrt{2n\sigma^2}}e^{\frac{[E-(E_{\mathrm{ab}}+nh\nu)]^2}{2n\sigma^2}}.$$

This expression defines, also the time-shape of the vibrational transitions occurring in multi-photon energy, nh$\nu$, where n is an integer [10]. In fact vibronic coupling takes place among a number of electronic energy levels that include multiple vibrational modes, coupled linearly. In $Er^{3+}$, doped in $NaYF_4$ crystals multi-photon vibrational bands were found to extend 1000 $cm^{-1}$ and above. Thus, multi-photon up-conversion of the $Er^{3+}$, doped in $NaYF_4$ crystals, that we used have a large contribution from vibronic transitions. Therefore, the vibrational frequencies found in the surface layer of the crystal are often different from the core frequencies. The relationship between crystal size and up-conversion emission wavelength and decay rate, is of high importance to up-conversion applications such as the ones described in this article that concern the wavelength extension of human vision.

To achieve this goal, we have used micro and nano particles which consist of a matrix of Sodium Yttrium Fluoride ($NaYF_4$) crystals doped with Erbium ($Er^{3+}$) and Ytterbium ($Yb^{3+}$) ions [11]. This material combination is known to be one of the most efficient materials for converting infrared ($\sim$ 980 nm) light to the visible ($\sim$540 nm and $\sim$650 nm) light which is easily detected by the rod and cone visual pigments, and in addition find a wide use in photodynamic therapy, solar cell research and other applications [12]–[15]. The development of these particles, since the 1960s, with significant advances in the 1970s and continuing improvements to the present date have resulted in the novel techniques that enhance the performance and efficiency of the up-converting particles. The $Er^{3+}$ ions, act as emitters while the $Yb^{3+}$ ions are the sensitizers. $Yb^{3+}$ absorbs IR light in the 980 nm region followed by the most efficient up-conversion mechanism which involves the transfer of energy from multiple excited $Yb^{3+}$ ions to $Er^{3+}$ ions within the same crystal, subsequently the $Er^{3+}$ excited ions decay to the ground state by emission of green and red light (Figure 1).

Two of the most common mechanisms of up-conversion processes are

*Mechanism 1*

$Yb^{3+} + h\nu(\sim 980\ \mathrm{nm}) \rightarrow Yb^{3+}*$

$2Yb^{3+}* + Er^{3+} \rightarrow 2Yb^{3+} + Er^{3+}** \rightarrow 2Yb^{3+} + Er^{3+} + h\nu$ ($\sim$540 nm/$\sim$650 nm)

*Mechanism 2*

$Yb^{3+} + h\nu(\sim 980\ \mathrm{nm}) \rightarrow Yb^{3+}*$

$Er^{3+} + h\nu(\sim 980\ \mathrm{nm}) \rightarrow Er^{3+}*$

$Yb^{3+} * + Er^{3+}* \rightarrow Yb^{3+} + Er^{3+} ** \rightarrow Yb^{3+} + Er^{3+} + h\nu$ ($\sim$540 nm/$\sim$650 nm)

$Yb^{3+}*$ represent $Yb^{3+}$ ions in their first excited state ($^2F_{5/2}$), $Er^{3+}*$ represent $Er^{3+}$ ions in their first excited state ($^4I_{11/2}$) and $Er^{3+}$ $**$ represent $Er^{3+}$ ions in their second excited state ($^4F_{7/2}$).

The mechanism of up-conversion in doped materials with rare earth ions proceeds via multiple pathways; primarily through energy transfer up-conversion, 2-photon absorption and excited state absorption by stepwise-2 photon absorption [1], [3], [16]. These processes proceed under different quantum efficiencies which range from $\sim$ $10^{-13}$ for two photon absorption (through a virtual level) to $\sim$ $10^{-6}$ for stepwise two photon up-conversion and $\sim$ $10^{-3}$ for energy transfer up-conversion for input intensities of 1 W/$cm^2$ [16], [17].

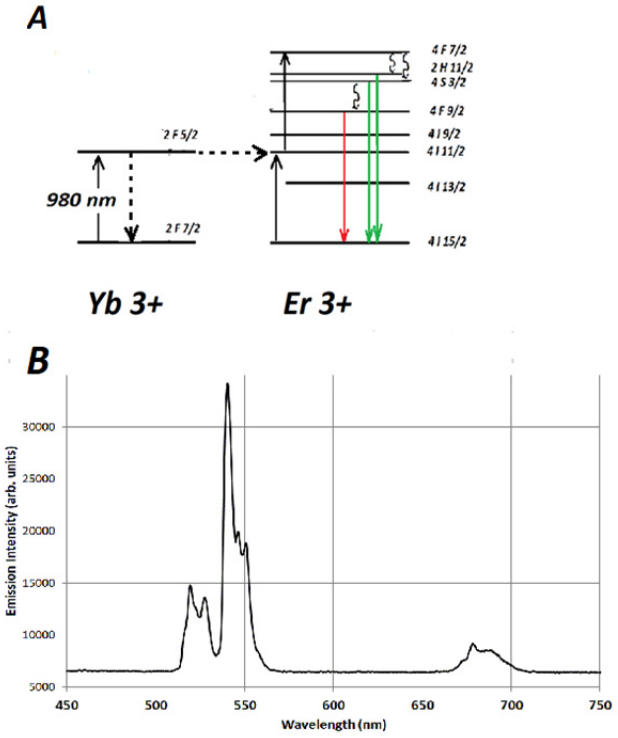


**FIGURE 1.** (A) Energy levels of $NaYF_4$:Yb,Er involved in the upconversion process and (B ) corresponding emission spectrum under 980 nm laser excitation.

It has been shown in [16], [17] that when the sensitizer ($Yb^{3+}$) concentration is much higher compared to emitter concentration ($Er^{3+}$), the energy transfer up-conversion mechanism, from the excited state sensitizer to excited state emitter ion, dominates and results in much higher up-conversion efficiency compared to a single ion two-step up-conversion through excited state absorption. Mathematical treatment of these up-conversion processes in [16] show that gain due to excited state absorption, compared to single ion step-wise absorption, is given by a factor of $N_s^2 W_{SA}^2$ where $N_S$ is the concentration of the sensitizer ions and $W_{SA}$ is the probability of energy transfer from sensitizer to emitter ion.

Conversion of UV light to visible is relatively simpler because many fluorescent molecules absorb in Near UV or UV region and emit at longer wavelengths, such as green light. A number of laser dyes may also be used and in fact a large number of bio-molecules are available which emit visible fluorescence upon absorption of UV light, two ssuch molecules are green fluorescent protein and yellow fluorescent protein.

For the conversion of ultraviolet light to visible, we have utilized Stilbene-420 dye and fluorescent proteins such as green fluorescent protein (GFP) owing to their unique absorption and emission properties which match very well with the spectral sensitivity of rods and cones of the human visual pigment.

## II. MATERIAL AND METHODS

The up-converting particles ($NaYF_4$:Yb,Er: 1 −5 micron average size) and proteins, were purchased from Sigma Aldrich. Stilbene-420 dye was purchased from Exciton and used without further purification. For fluorescence measurements, a Shimadzu RF-5301PC spectrofluorophotometer was used, while the absorption spectra were recorded by means of Shimadzu UV-160U spectrometer. The rod and cone cells used were extracted from bovine retina following the procedure described in [18]. For qualitative measurement of upconversion spectra (Figure 1), a USB spectrometer was utilized. For the approximate estimation of quantum efficiencies, a spectrometer system attached to a confocal microscope from HORIBA instruments was utilized. A Molectron (PM3Q with EPM1000) optical power meter was also used to measure optical power.

## III. EXPERIMENTAL RESULTS

### A. OPTICAL PROPERTIES OF UP-CONVERTING PARTICLES

The up-converting particle size used in our experiments, ranged from 1 um to 5 um, are of relatively larger size particles because as mentioned in a previous section, it has been shown [19], [20] that the quantum efficiency of the up-conversion process of $NaYF_4$:Yb,Er particles increases with increase in the average size; with micron size particles having several times higher quantum efficiency than nano size particles. The absorption spectrum of the up-converting particles exhibited a prominent absorption band centered at 976 nm, and their emission intensity, as a function of 980 nm excitation intensities is shown in Figure 2 (A).

A clear quadratic dependence of emission intensity with respect to excitation intensity can be observed in Figure 2 (A), which also shows that the quantum efficiency increases linearly with excitation light intensity, for the intensity ranges used ($\sim$ 100 mW/cm$^2$). This observation is consistent with earlier reported data [19]–[22] which also show that the quantum efficiency of these particles increases linearly with increasing excitation power density, before gradually reaching saturation as the excitation power densities reach a few Watts/cm$^2$. At this saturation regime, the quantum efficiency is reported to be 3-10 % for micron sized particles [19], [20]. Quantum efficiency calculations that compared up-converting particles emission intensity with a dilute solution of Rhodamine 6G dye in methanol revealed a quantum efficiency of $\sim$ 0.5% for a power density of 0.15 mW/cm$^2$ for the micron size particles. The calculation of quantum efficiency was made by using a Spectrometer, attached to a confocal microscope. A dilute solution of Rhodamine 6G dye (0.005 mg/mL), in methanol, was used as a reference. This solution, was excited with a known power of 532 nm light (25 $\mu$W), in backscattered geometry, a 0.25 NA microscope objective was used to record the instrument response function. The same system was also used to measure the emission from an up-converting particle suspensions under a 980 nm excitation. This emission, the up-converted emission was compared with the Rhodamine 6G emission to arrive at the estimated value of 0.5 % quantum efficiency of the up-converting particle suspension. Figure 2 (B ) shows the dependence of up-converted emission on the

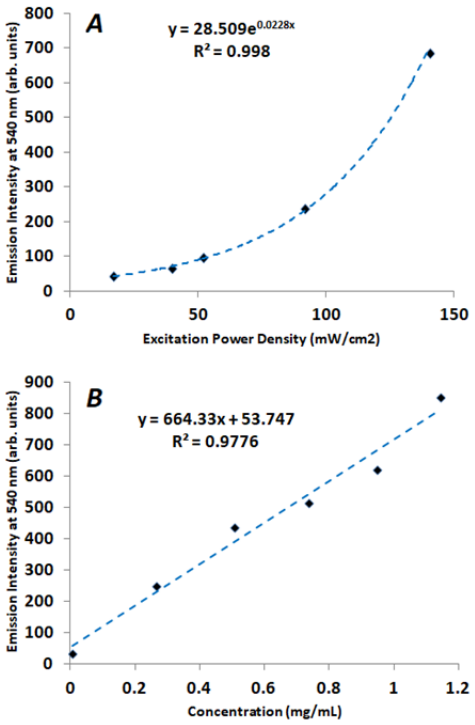


**FIGURE 2.** **(A) Upconversion emission intensity at (540 nm) versus input power density (at 980 nm), [Conc. of upconverting particles: 0.8 mg/mL in Water; particles size 1-5 um] and (B) Concentration of upconverting particles dispersed in water versus the emission Intensity(at 540 nm), excited at 980 nm with 140 mW/cm$^2$.**

concentration of the particles dispersed in water. For concentrations upto 1 mg/mL the plot, as expected is linear.

### *B. UP-CONVERTING PARTICLES DISPERSED IN PROTEINS*

Up-converting particles were mixed with various protein solutions, including Bovine Serum Albumin (BSA), Egg albumin, Whey (milk) protein and green fluorescent protein (GFP) and their up-converting properties were studied. Figure 3 shows that the up-conversion behavior of the up-converting particles in water and protein solutions are very similar. All the materials were excited with 980 nm, 130 mW laser beam. An advantage of the dispersion of upconverting particles in proteins is their possible introduction into the eye without deleterious effects on the eye or visual process.

### *C. UP-CONVERTING PARTICLES MIXED WITH VISUAL PIGMENTS*

The bovine retina rod and cone cells were isolated using a previously described procedure [18].

These visual pigment cells were suspended in Tris buffer, and irradiated with 532 nm and 980 nm light. To the mixture which was irradiated with 980 nm infrared light, $NaYF_4$:Yb;Er micro particles were added, at a concentration of $\sim$ 0.9 mg/mL. The bleaching rate (due to absorption of green light) of the visual pigment in rod cells was calculated by measuring the change in optical density of the 500 nm band of rod cells. When rod cells were irradiated with visible

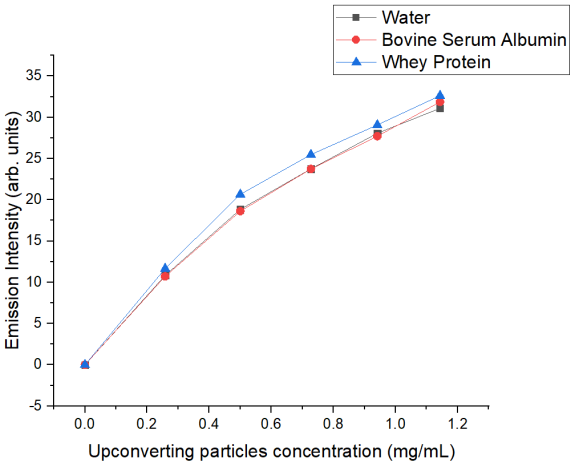


**FIGURE 3.** **Intensity of upconverted emission versus concentration of particles in water and protein mixtures. The concentration of both proteins $\sim$ 0.5mg/mL**

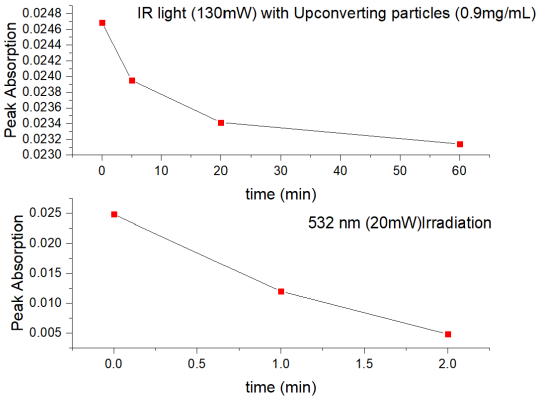


**FIGURE 4.** **Bleach rate of visual rod pigment cells with visible and infrared light (added with upconverting particles).**

or infrared light (in the presence of $NaYF_4$:Yb;Er particles), the 500 nm rhodopsin absorption band intensity decreased gradually. However, there was no decrease in the 500 nm band when rods were irradiated with infrared light without up-converting particles present; this suggests strongly that the up-converted green emission induces vision.

Figure 4 shows the comparison of the bleaching rate under 532 nm visible and 980 nm infrared excitation in the presence of up-converting particles. The observed bleaching rate under 980 nm, 130 mW laser light was roughly three orders of magnitude slower than the 532 nm bleaching rate. Both the green and Infrared lasers irradiated $\sim$ 10 mm$^2$ area of the cuvette containing $\sim$1.5 mL volume of the samples which were continuously stirred.

### *D. ULTRAVIOLET TO VISIBLE CONVERSION*

In order to convert ultraviolet light to visible, Stilbene-420 dye was used which emits intense blue fluorescence upon irradiation with ultraviolet light (Excitation from 330 nm to 380 nm). The Stilbene-420 dye emission has a maximum at 425 nm which, almost perfectly, overlaps with the absorption

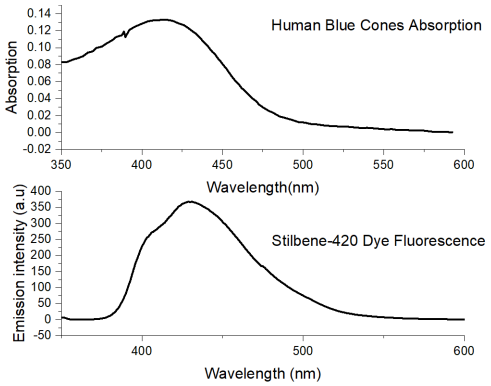


**FIGURE 5.** Absorption spectrum of human blue cones (upper, adapted from [25]) and Emission spectrum of Stilbene dye (lower).

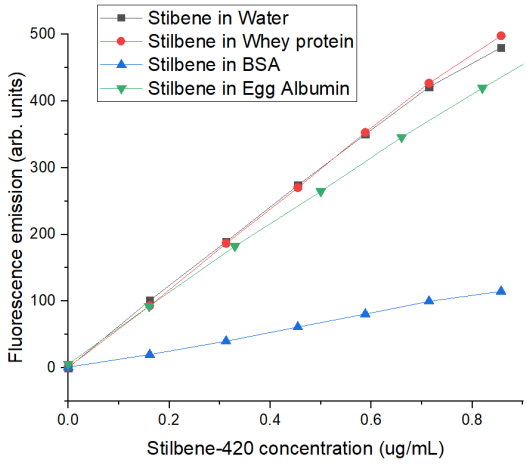


**FIGURE 6.** Stilbene-420 dye fluorescence emission intensity versus concentration (in water & egg ovalbumin protein solution [protein conc. ~ 0.5 mg/mL]), [excitation wavelength: 350 nm].

spectrum of the human eye blue cones (Figure 5). Blue light emission is also known to help regenerate the bleached visual pigments [23], [24]. In our experiments, we found that when stilbene-420 dye is mixed with proteins, such as Egg albumin, bovine serum albumin, and whey (milk) protein etc. The Stilbene dye maintained its fluorescence efficiency and there was no detectable quenching of the fluorescence intensity due to the presence of the proteins (Figure 6) except for the bovine serum albumin solutions, where we observed a decrease in the fluorescence emission intensity of stilbene by a factor of 4 to 5. We have not yet investigated the causes for this decrease although it seems likely that is due to the interaction of Stilbene with the amino acids with thiol groups such as methionine and cysteine resulting in change in the excited state dipole of Stilbene as has been suggested [35].

While Stilbene-420 dye is a very promising candidate for ultraviolet to visible conversion, its absorption maximum is around 350 nm. Human eye lens and tissues however strongly attenuate the UV light below 390 nm, therefore stilbene-420 will not be able to absorb much of the UV light unless it is deposited on top of the eye lens. However, it can still

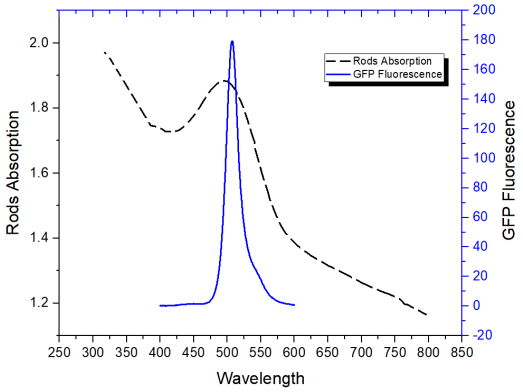


**FIGURE 7.** Fluorescence emission spectrum of green fluorescent protein, excited at 390 nm and absorption spectrum of bovine rod cells.

be used in a wearable optical device which is described in section IV of this paper.

Very attractive alternatives to Stilbene-420 dye for insertion in the eye are naturally fluorescent proteins such as green fluorescent proteins (GFP) which can be used to absorb UV and convert it to visible green light. The absorption spectrum of Green Fluorescent Protein (GFP) can be tuned to the near UV light [26] thereby making it a suitable biological compound for near UV absorption and conversion to visible light. The absorption spectrum of GFP shows a maximum at 390 nm and a weak absorption band at 475 nm, the emitted fluorescence spectrum overlaps with the 500 nm region of mammalian rod cells sensitivity (Figure 7).

### *E. COMBINING UPCONVERTING PARTICLES WITH DOWN CONVERTING PROTEINS*

We observed that upon addition of up-converting (IR to visible) particles to the down-coverting (UV to visible) fluorescing protein solutions (BSA-Stilbene solution & GFP solution), both, the up-converting micro-particles and the down-converting protein retained their spectroscopic properties and therefore we used this mixture to convert both UV and Infrared light to visible light and plan to deposit them in the eye of animals such as a rabbit.

Figures 8 & 9 show the fluorescence emission from the green fluorescent protein (gfp) & upconverting particles mixtures; and Stilbene & up-converting particle mixtures under UV and near-infrared excitation, respectively. The concentration of the green fluorescent protein and up-converting particles was $\sim 0.05$ mg/mL and $\sim 0.4$ mg/mL, respectively. We established experimentally that the fluorescent behavior of GFP remained unchanged after the addition of up-converting particles.

## IV. BROADBAND VISION GLASSES

In this section we describe the design and present the data recorded with an optical device which utilizes both the up-converting particles and fluorescent proteins/dyes, to extend the spectral range of human vision to UV and IR. This optical system consists of two lenses and a screen

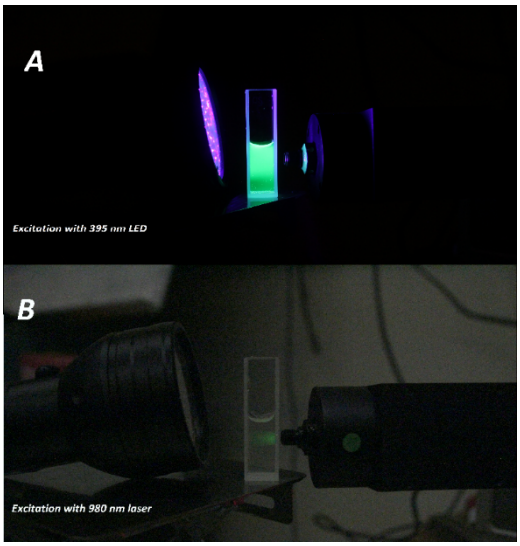


**FIGURE 8.** (A) Fluorescence emission of green fluorescent protein (GFP) and up-converting particles mixture under near UV light (395 nm) and (B) under Near infrared (980 nm) excitation.

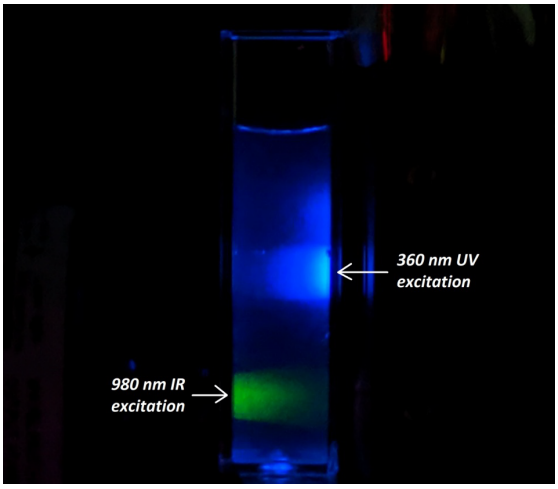


**FIGURE 9.** (Upper) Fluorescence emission of Stilbene dye, ovalbumin protein and upconverting particles mixture under near UV light (360 nm) and (Lower) under Near infrared (980 nm) excitation.

placed at the image plane of the objective lens coated with up-converting particles. The image formed by the environmental infrared light, i.e sunlight, is imaged on the screen where the infrared light image is converted into a visible image by the upconverting particles and is observed by a normal eyepiece lens. This system can be easily made compact by utilizing a mirror in place of objective lens. The use of mirror also helps to eliminate chromatic aberrations as it will focus all wavelengths to same point.

In one version of this device the central up-converting screen is composed of a set of thin layers of up-converting micro-particles and layers of UV to visible converting fluorescent dyes doped in glass. Figure 11 shows an image recorded by an eyepiece under visible and 980 nm infrared illumination where the up-converting screen consists of a glass slide coated with the $NaYF_4$:Yb,Er up-converting

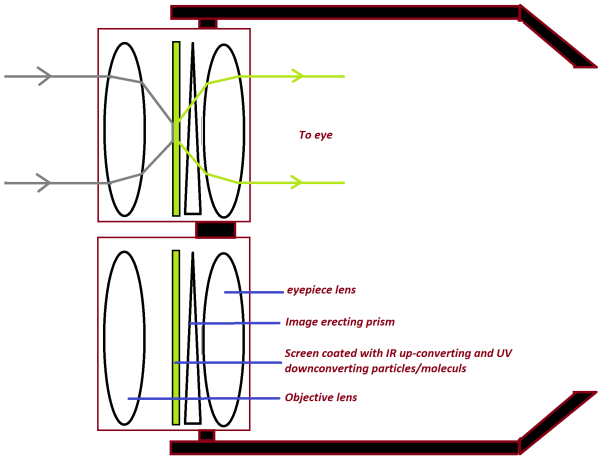


**FIGURE 10.** Basic design of the upconverting particles & fluorescent dye based IR and UV vision device.

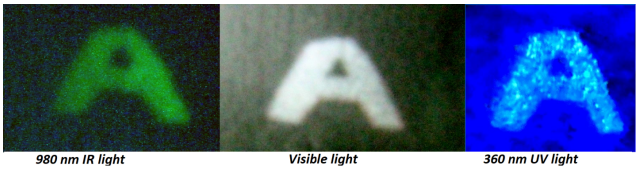


**FIGURE 11.** Images recorded through the designed optical device under infrared, visible and ultraviolet illuminations.

particles. The power density of the 980 nm infrared light impinging on the sample was $\sim$ 500 mW/cm$^2$ whereas the power density of visible light illumination was $\sim$ 1mW/cm$^2$. The picture was taken by placing a camera behind the eyepiece, where the eye would be located.

## V. DISCUSSION AND FURTHER EXPERIMENTS

We expect that up-converting particles may enable the eye to detect the invisible to human eye infrared light, however, the infrared light intensity at the present has to be significantly higher, 2-3 orders of magnitude than visible light owing to the low two photon (Stepwise) absorption efficiency (cross-section). This is valid for both, our eyeware optical devices and for biologically administered up-converting particles. This large intensity difference was also observed, recently, when researchers injected similar nanoparticles in mice eye in order to enable vision in the infrared [27]. Owing to the large dynamic range of the eye, detecting infrared light with these particles is very promising owing to the fact that rods can detect a single photon and recognize a few photon ($\sim$100) image [28]. The challenges remaining to be overcome, for widespread use of such techniques include a.) improving the relatively low up-conversion efficiency, in order to detect lower IR intensities and b.) increasing the narrow wavelength absorption band in the infrared region of the particles to wider wavelength range.

In order to address, both of these issues, namely narrow-band absorption and low quantum efficiency at low power densities, promising research is being carried in the area

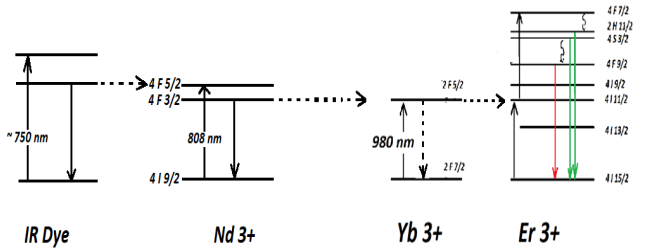


**FIGURE 12.** Energy level diagram of IR dyes transferring energy to $Nd^{3+}$ (808 nm) and $Yb^{3+}$ with energy transfer to $Er^{3+}$ for two photon up-conversion of IR to visible wavelengths.

of core shell structures of these nano-particles. Such particles can be doped with a shell of additional ions such as $Nd^{3+}$ ions which provide additional absorption bands near 800 nm corresponding to $Nd^{3+}$ ions absorption. Fluorescence emission from $Nd^{3+}$ ions in turn excite $Yb^{3+}$ ions resulting in up-converted visible emission [29]–[31]. The efficiency of the fluorescence energy transfer from $Nd^{3+}$ to $Yb^{3+}$ is shown to be very high (∼70%)[32]. It is also possible to design organic dyes which sensitize up-converting particles, in this case an additional layer of IR absorbing dye is grown on top of the upconverting micro-particles resulting in an appreciable increase in infrared light absorption efficiency owing to the higher cross section of these IR absorbing dyes [29], [32]–[34]. Figure 12 depicts the Energy levels of Nd3+ and dye sensitized core -shell up-converting particles that we are considering. In addition to these methods, traditional broadband upconversion techniques, such as, Second Harmonic Generation (SHG) in non-linear crystals, or two photon fluorescence, may also be utilized if the intensities of light used are high, such as with pulsed lasers. However, at lower power densities of a few hundred $mW/cm^2$ to a few $W/cm^2$, efficiency of traditional techniques is extremely low.

## VI. CONCLUSION

We have utilized up-converting micro-particles doped with $Yb^{3+}$ and $Er^{3+}$ to convert 980 nm near infrared light to 540 nm and 650 nm visible light, thereby extending the human vision range to the near IR region. In addition, we have been able to use fluorescing protein solutions to convert UV to visible light. We have also combined the IR and UV to visible converting micro-particles and proteins to initiate visual processes in rhodopsin visual pigments.

In addition, we describe the design and construction of an optical system which enables the wearer to see intense UV and near IR images.

## ACKNOWLEDGMENT

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