# Color matching of two-photon stimuli projected by scanning laser

### Mateusz Grochalski<sup>a,\*</sup>, Magdalena Smolis<sup>b</sup>, Elena Moreno<sup>c</sup>& Katarzyna Komar<sup>a,d,e,#</sup>

<sup>a</sup>Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Toruń, Grudziądzka 5, 87-100 Toruń, Poland

<sup>b</sup>Institute of Physics, Jagiellonian University in Kraków, Łojasiewicza 11, 30-348, Krakow, Poland <sup>c</sup>Faculty of Optics, Complutense University of Madrid, Calle de Arcos de Jalon, 18, San Blas-Canillejas, 28037 Madrid, Spain <sup>d</sup>International Centre for Translational Eye Research, Skierniewicka 10a, 01-230 Warsaw, Poland <sup>e</sup>Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland <sup>#</sup>corresponding author email: kkomar@fizyka.umk.pl \*Presenting author

## 1. Main text

Human perception of light is traditionally considered to be restricted to so-called visible range of spectrum – approximately 400-700 nm. However, over several decades of working with pulsed infrared lasers, various observers have reported visual sensation when exposed to beams from outside the visible range [1]. The observed color of beams in the range of 850-1200 nm corresponds to one-half of the IR wavelength. This phenomenon was later explained by two-photon absorption (TPA) occurring when visual pigments present in photoreceptors interact with pulsed laser beams [2]. TPA occurs when two photons are simultaneously absorbed (within  $10^{-18}$  s [3]) by the pigments [1]. Requirement of such simultaneity explains why TPA is much less probable compared to single-photon absorption (SPA). Detecting an infrared beam with human eye thus requires high laser power, which pulsed lasers can provide by delivering large peak power over short duration while maintaining average power within safety limits.

The percentage of photons that get absorbed by TPA and SPA varies on the laser power, since probability of TPA is proportional to square of average laser power, whereas probability of SPA increases linearly [4]. Consequently, as laser power increases, there is a shift in color perception due to a different proportion of TPA, leading to a different amount of red-shift. Our goal was to check if gathered data would support this hypothesis.

Another goal was to compare our results with data from Gil et. al. [5]. For color matching they used a display with a gamut that didn't contain colors corresponding to monochromatic colors, particularly in the green region. It resulted in strong deviating of IR points at around 1000 nm from monochromatic colors. They discuss this limitation in their conclusions. Our study, however, is using a better suited gamut that covers more of the color space. We verified that the color matching for visible stimulus (520 nm) closely aligns with the actual wavelength, suggesting that our color determinations for IR are probably closer to reality. One more factor that explains differences between our results and published by Gil et al. is brightness of two-photon stimuli resulting from average power level, duty cycles of both lasers [4] and stimuli size. By taking into account these differences, our two-photon stimuli were 5-200 times brighter than used in Gil et al. [5].

A full understanding of two-photon color perception will broaden knowledge of two-photon vision and could be useful in development of two-photon retinal display [5].

## 2. Methods and results

The first task was to achieve two similar square stimuli drawn by scanning laser – one stimulus was drawn by visible (VIS) laser (520 nm), and one by infrared (IR) laser (1040 nm), both were emitted by HighQ "FemtoTrain" laser with 76 MHz frequency, and 260 fs pulse width. Angular size of both stimuli was  $\sim$ 0,83° and they were presented side by side. They consisted of 30 horizontal lines, which was enough for both stimuli to be perceived as homogeneous.

The diagram of optical system is presented on Fig 1A. Lens L9 was used for refractive error correction. Lenses L1 and L6 corrected chromatic aberrations between stimuli of different wavelengths. The eye was accurately positioned in respect to the optical axis of the system using infrared camera (CAM) that was optically coupled with the pupil plane. The first task of four participants was to match the brightness of the VIS laser to IR one. The matching was done for four different values of IR power:  $800\mu$ W,  $600\mu$ W,  $400\mu$ W,  $200\mu$ W at pupil plane (altered with gradient filter G2). For each power level, data was collected 5 times for averaging purposes. Participants could freely change the power of VIS stimulus, by rotating the gradient filter G1 using mouse scroll wheel with a step of 3 degrees, what roughly corresponds to 0,5 dB. After matching the power values for both stimuli, the color matching procedure began.

Light from 4-Wavelength High-Power LED Head (LED4D008) was coupled into single-mode optical fiber. The output fiber tip with the pivot of galvanometric scanners (GS), what resulted in drawing another square-like homogeneous stimulus of tunable color. LED driver allowed for independently changing current level for red (655 nm), green (520 nm), and blue (450 nm) LEDs by a step of 1 mA. The task of participants was to match the color of LED stimulus to IR and VIS stimuli. Spectrum of LED light was gathered using spectrometer (AvaSpec-ULS2048XL-EVO).

The color matching functions [6] were used to calculate corresponding coordinates on standardized CIE1931 color space.

Results are shown at Fig 1B. Data gathered by Gil et. al. are also plotted for comparison as black symbols. It is apparent, that by increasing the power of stimulating laser, IR data points become more and more red-shifted, while VIS data points stay in the same place. The referenced data show this trend too, however it is less apparent, because their display has different color gamut (black triangle) than our source of visible light (gray triangle).

As suggested in [6], the perceived hue dependence on power appears to be the result of the linear versus squared dependence on intensity of 1P and 2P absorption, which results in different percentage of L-cones and M-cones that are being activated. Although the effect is more apparent for shorter wavelengths near the border of the visible spectrum, it is still noticeable for 1040 nm and 1050 nm. Using High-Power LED Head (LED4D008) resulted in more broad gamut, which helped in acquiring more accurate data closer to the monochromatic locus.

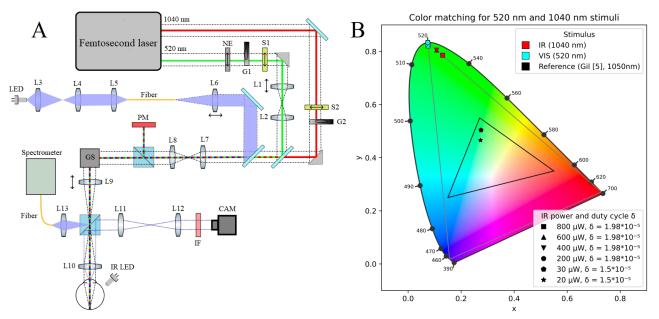


Fig 1: A) Optical system. L - lenses, S - shutters, NE - neutral density filter, G - gradient filter, GS - galvanometric scanners, IF - IR mirror (pointing upwards), PM - power meter,

B) CIE 1931 color pallet with plotted data points. "x" and "y" are the chromacity coordinates. Each point's coordinates are the average of all participants' for a given power. Triangles represent gamuts: gray – LED4D008 display, black – AMOLED display from Gil et. al. [5]. The 200µW data point for IR stimulus lies on monochromatic locus (520 nm) and is covered by VIS data points. The power values of VIS stimulus corresponding to IR powers are on average: 296pW, 150pW, 77pW and 21pW for 800µW, 600µW, 400µW, 200µW respectively.

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