# The Setup for the "Visual Pigment 544" Researches Based on Contact Interferometer (Uverskii, 1958-1969) with $\lambda$ =544 nm

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#### **Abstract**

"Visual pigment 544" as a presumptive cone pigment from the retina is well known from 1960th. Spectral maximum for M-type cones is ≈ 544 nm. Therefore, it is rational to implement a spectral and electrophysiological measurement system for visual science with the possibility of using a 544 nm wavelength. We propose to use a contact interferometer (for example, classical Uverskii-type interferometer with a 544 nm filter) for this purpose. Contact interferometers have been a classic research and quality control tool since the 1960s. Currently, they are equipped with computer vision systems with CMOS cameras and software for automated image analysis.

Keywords: Visual Pigment 544; Contact Interferometer; Uverskii Interferometer; M-Type Cones.

## 1. Introduction

"Visual pigment 544" as a presumptive cone pigment from the retina is well known from 1960<sup>th</sup>, since the classical "Nature" article (Bridges, 1962) actively cited in 1960<sup>th</sup> – 1980<sup>th</sup>, including some basic handbooks on sensory physiology and biochemistry of visual processes. This article has generated much discussion and has been cited in particular in the famous pioneering work "Are colour oil droplets the basis of the pigeon's chromatic space?", which also was often cited in 1970<sup>th</sup> – 1980<sup>th</sup> [1-17].

Now it is well-known, that S-type cones are sensitive in violetblue spectral band (short-wavelength spectrum), M-type cones are sensitive in green-yellow spectral band (medium wavelength), and L-type cones are sensitive in yellow-red spectral band (long-wavelength). Spectral maximum for S-type cones is  $\approx 443$  nm. Spectral maximum for L-type cones is  $\approx 570$  nm. Spectral maximum for M-type cones is  $\approx 544$  nm. Therefore, it is rational to implement a spectral and electrophysiological measurement system for visual science with the possibility of using a 544 nm wavelength.

In the paper we can read: "We have therefore determined action spectra for light induced cone contractions in the retina of the dichromatic cichlid fish, Aequidens pulcher, which has redsensitive double cones with a  $\lambda_{max}$  of 617 nm and green-sensitive single cones ( $\lambda_{max}$  544 nm) in addition to a rod pigment absorbing maximally at 501 nm" and "These action spectra have been fitted to the visual pigment absorption spectra of ... single cones ( $\lambda_{max}$  544 nm, optical density 0.3)" [18].

In some cases, the wavelength of 544 nm is the peripheral or

boundary, and not the central one for a given spectral band. For example, the authors of the paper write: "For quantitative estimation of chromophore proportions, we considered mainly rods and M-cones [19]. In four populations, spectra of both photoreceptor types 525–530nm for rods and 535–544 nm for M-cones)". And more: "For M-cones, the range of □max population means was 519–544nm, well encompassed by the DLH relationship and thus consistent with a single opsin. However, the 25 nm range is so narrow that only one (or neither) of the boundaries can represent a pure chromophore version of the pigment".

Sometimes such distributions are bimodal. For example, in the paper authors write: "The records from the cones ... also had a significantly bimodal distribution (d.i. = 256.91, P < 0.01), with a  $\lambda$ max of 544 nm for one group and 564 nm for the other" [20]. And they refer to the Jacobs work: "Jacobs (1990) has demonstrated a notch near 580 nm in a female callitrichid (Saguinus fuscicollis) with putative pigments at 544 and 557 nm" [21].

Many sophisticated experiments and setups were constructed and designed for the registration of action spectra of photomechanical cone contraction in the retina. But it is obvious that the most popular schemes of such setups and experiment geometries are not optimized for microstructural, cytophysiological and histological investigations. For example, in the paper "For each experimental exposure, two completely dark-adapted fish were placed in the adapting aquarium and subjected to a particular combination of wavelength and intensity for 45 min [22]. The order of presentation of the test wavelengths (443,

496, 544, 620 nm) and intensities was random". The authors inevitably conclude: "Unfortunately, the histologic sections obtained for the fish exposed to the second highest intensity of 544 nm stimulation were not good enough to allow an accurate determination of the cone index". Consequently, it is necessary to develop installations for exposing the eye by above mentioned wavelengths under microscopic (time-lapse or other) registration.

Now it is obvious, that the basic technique for such investigations is the analysis of the cone photo responses obtained with electroretinogram from the isolated retina under the microscope. Some works in this methodological trend are accented on the 544 nm wavelength as optimal  $\lambda$  for the isolated retina stimulation. For example, in the paper we can see that "...the stimulus wavelength (544 nm) was chosen to selectively stimulate the green sensitive ("M") pigment [23]. Obtained responses were monophasic, showing fast kinetics (mean tp = 51 ms) and low sensitivity (fractional single-photon response ca. 0.23%)". The classic and fundamental work for this wavelength (544 nm) is the article where the 544 nm laser was used [24]. In this classical source we can see, that "the focal cone electroretinogram (ERG) in monkey retina has been examined with a 3 deg pulse of laser light (544 and 633 nm) centered on a 25 deg steady white rod saturating field" and "two interchangeable helium-neon lasers, 544 and 633 nm" were used. In this experiment:

- "The foveally centered cone ERG to the red (633 nm) and green (544 nm) laser stimuli at different relative intensities indicated on the left by the amount of neutral density filtering interposed in the laser beam"
- "Foveal cone ERGs to the 633 and 544 nm stimuli at different flickering rates. The upper trace of each set shows the response to the 633 nm stimulus and the lower to 544 nm stimulus. Numbers on the left side of each set indicate flickering rates (Hz)".
- "The implicit time to the 633 nm stimulus is later than it is to the 544 nm stimulus at the fovea, but this difference disappears

- at 10 deg from the fovea".
- "The response at the fovea is larger with a later peak latency to both 633 nm (red) and 544 nm (green)".
- "With a full-field stimulus the 544 nm stimulus also produces a quicker response than the 633 nm stimulus and this difference is independent of the amplitude".

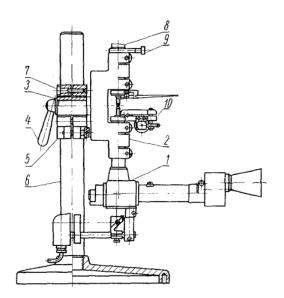
Theoretically, different lasers can be used and different types of installations can be built on their basis, such as:

- 1. HeNe lasers, frequently used in flow cytometry, immunofluorescence and fluorescence microscopy and molecular genomics [25,26].
- 2. Dye lasers, including fluorescein-based dye lasers [27].
- 3. Diode-pumped lasers, including modern Yb:GSO-LBO and Nd:YVO4-LBO lasers and "classical" diode-pumped doped glass fiber lasers [28-30].
- 4. Other complex fiber lasers, including diode-oscillator fiber-amplifier potentially usable for calcium spectroscopy [30-33].
- 5. Second harmonic generation laser systems [34,35].
- 6. Q-switched Tb: LiYF4 green lasers [36,37].

But it is obvious, that such laser sources can be very expensive. Many such lasers (with high energies in the beam or pulse) cannot be considered as non-destructive testing tools for visual science and human-oriented ophthalmology. Consequently, a low-intensity light source with variable power is needed for microscopic observations in ERG-assisted retina investigations.

# 2. Towards the Optical Setup Construction

We propose to use a contact interferometer (for example, classical Uversky-type interferometer (see Fig. 1, Fig. 2) with 544 nm filters for this purpose [37-39]. Contact interferometers have been a classic research and quality control tool since the [38,40-42]. Currently, they are equipped with computer vision systems with CMOS cameras and software for automated image analysis [43].



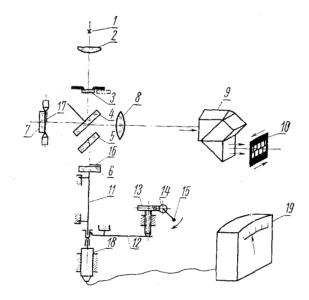


Figure 1: Uverskii interferometer (from Izmeritel'naya Tekhnika, No. 1, pp. 29-30, January, 1968) [38].

The contact interferometer is designed to measure linear dimensions by the comparative method. Its action is based on the principle of two-beam interference of light. The contact interferometer manufactured by the KALIBR factory has a green source (544 nm) with a variable intensity. The main unit of both types of interferometers is the interferometer tube. A distinctive advantage of the contact interferometer is a device for changing the scale division value in the range from 0.05 to 0.2 µm. According to the instructions for the instrument, the vertical

contact interferometer has rigid cast bases and a stand. A bracket carrying the interferometer tube can be moved along the rack guide with the help of a rack. A specialized screw allows you to shift the scale of the tube within 10 divisions. A heat shield is attached to the tube. The interferometer table (on which the sample can be placed and irradiated with an appropriate light source) can be moved vertically with a micro-feed screw or locked in position with another screw.

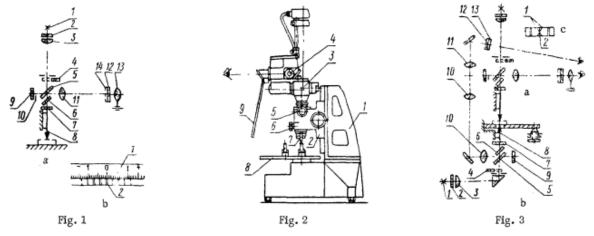


Fig. 1. (a) Diagram of the ocular (screen) tube. 1) Light source; 2) heat filter; 3) condenser lens; 4) light filter; 5) beam divider; 6) compensator; 7) movable mirror; 8) measuring rod; 9) basic mirror; 10) shield; 11) objective lens; 12) scale; 13) ocular [or 14) screen]. (b) Field of view on the scale. 1) Sample scale; 2) guide.

Fig. 2. 1) Stand; 2) support; 3) ocular tube; 4) scale adjustment; 5) microadjustment tube; 6) stop; 7) holder; 8) fixed stage; 9) shield.

Fig. 3. (a) The same as in Fig. 1a. (b) Special tube. 1) Light source; 2) heat filter; 3) condenser lens; 4) light filter; 5) beam divider; 6) compensator; 7) movable mirror; 8) measuring rod; 9) basic mirror; 10) objective lens; 11) microobjective; 12) screen; 13) grid. (c) Field of view on the grid. 1) Grid lines; 2) guide.

Figure 2: Uverskii Interferometer (from Izmeritel'naya Tekhnika, No. 3, pp. 17-19, March, 1969) [39].

So, contact interferometry or contact micro interferometry very well correlates with methods of contact microscopy in biology known since the 1950s and often used in particular for ophthalmology [44-54]. Thus, the application of the method of contact microscopy, in the particular case of contact interference microscopy or contact interferometry, is not limited to technical (in particular, tribological aims or strength measurement tests, but is included in the field of biology of sensor systems and visual science [55-58].

## 3. Technical Remarks

The simple device for microinjections, manipulations and measurements using an electro-morphological chip under micro interferometric control of the interface and membrane processes at the thickness range of 5-1000 nm at the different electrode angles for such experiments described in the paper [59]. But it can not be used with classical Uverskii-type contact interferometers. Despite this fact, such measurements can be implemented on the MII-11 (micro)interferometer with interference filters with adjustable  $\lambda$ =544 nm. Example of the setups configurable with interference filters with adjustable  $\lambda$ =544 nm on MII-11 micro interferometers can be viewed on the Fig. 3 (a, b).





**Figure. 3:** The Simple Device for Microinjections, Manipulations and Measurements Using an Electro-Morphological Chip Under Micro Interferometric Control of the Interface and Membrane Processes (at the thickness range of 5-1000 nm at the different electrode angles) based on MII-11 Micro Interferometer.

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