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Spectrozonal/Multispectral Analysis of Ascaris Lumbricoides Based on Coherent and Incoherent Light Sensor LLM Platforms

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Abstract

As global temperatures rise and ecosystems shift, the geographical range of many parasitic worms may expand, leading to increased risks of transmission to new populations. Microscopic analysis allows scientists to monitor these changes and anticipate potential disease outbreaks, enabling timely interventions to protect public health and agricultural productivity. The implementation of affordable lensless microscopes holds immense importance for parasitological control in African and developing countries. These innovative tools offer a cost-effective solution for conducting microscopic examinations, particularly in resource-limited settings where traditional microscopes may be inaccessible or expensive. Of special significance is the potential of laser-based lensless microscopes to replace confocal microscopy methods for studying both human parasites and parasite eggs found in the natural environment. By utilizing these advanced technologies, researchers and healthcare professionals can enhance their ability to accurately identify and analyze various parasitic organisms, contributing to more effective disease surveillance and control efforts. In African and developing countries where parasitic infections pose significant health challenges, the adoption of lensless microscopes can revolutionize diagnostic capabilities and research outcomes. These portable and easy-to-use devices empower local healthcare workers and scientists to conduct rapid and precise examinations, leading to timely interventions and improved public health outcomes. By leveraging the advantages of lensless microscopes, such as affordability, portability, and versatility, these countries can strengthen their parasitological control strategies and advance towards sustainable healthcare systems. The integration of cutting-edge technology in parasitology not only enhances disease detection and monitoring but also fosters scientific innovation and capacity-building within local communities. By harnessing the potential of these innovative tools, we can revolutionize the way we study and combat parasitic diseases, ultimately improving health outcomes and promoting sustainable development in resource-constrained regions.

Keywords: Ascariscope, Ascarimeter, Lens-Less Microscope, Lens-Less Holography, Electronic Speckle Pattern Imaging (ESPI), Spectrozonal Imaging, Multispectral Mapping, Colocalization

1. Introduction

It is well known that alongside highly advanced techniques, available only to a small number of users, such as analytical electron microscopy and laser microscopy and microdissection, standard routine optical microscopy methods are actively used in parasitology [1,2]. These methods are commonly employed for various mass-scale tasks, such as the analysis of fecal biomaterial for indicators of invasions, including the identification of eggs of Ascaris lumbricoides, Dicrocoelium dendriticum, Schistosoma sp., hooks for Echinococcus granulosus, nematode larvae, Strongyloides stercoralis, where the use of even elementary means of research microscopy (special types of contrast, fluorochrome staining, etc.) is generally considered excessive. Furthermore, the focus for equipment manufacturers is often not on increasing the number of diagnostic descriptors but on simplifying equipment for mass analysis. This has led to the development of parasitological or helminthological virtual microscopy systems and tools particularly those based on web interfaces in the digital era, moving towards mass telemedicine [3-6].

At the beginning of this trend, combined devices for telemedicine parasitological diagnostics were essentially conventional microscopes with simplified optical systems equipped with a webcam. Subsequently, with the advancement in the resolution of light-sensitive matrices and the reduction in pixel size, a transition to lensless microscopy was made. Lensless microscopy became so cost-effective that Asian and African countries, where developed microscopy had previously been a significant material and organizational obstacle to implementing microscopic screening in mass parasitic diagnostic protocols, were able to afford its adoption. A leading advocate for democratizing diagnosis based on lensless microscopy is Aydogan Ozcan from UCLA. Morphometric diagnostic patterns relevant to parasitology, such as those for trypanosomes (causative agents of sleeping sickness) and malarial plasmodium, were researched and included in databases for machine learning (supervised learning). Additionally, morphological practices for free-living roundworms involved the study and identification of free-living nematodes of the species Caenorhabditis elegans [7-13]. Machine learning methods were implemented using volunteers based on packages like BIOGAMES designed for this purpose [14,15].

It is worth noting that volumetric/pseudo-volumetric representation of helminthological and parasitological objects was achieved through the use of holography on a chip and laser multi-angle projection technologies. Subsequently, defocusing and the need for Y-positioning using a fine-tuning handle were eliminated due to contact localization and software capabilities [16]. Record exposure times were achieved using terahertz or femtosecond laser techniques [17]. However, in general, measurements of this kind and the registration of pseudo-three-dimensional holographic patterns and parasite patterns in laser Schlieren methods and speckle-mediated projection methods do not require such exotic technologies and can be implemented using lasers from ordinary laser pointers.

Therefore, even for field research conducted in extremely low-budget conditions, carrying out lensless microscopic and holographic studies on a chip does not pose a significant problem. We conducted a comprehensive set of studies for previously unexplored representatives of the Ascaridae family in a sectioned state, which hold particular parasitological significance in the case of Ascaris lumbricoides.

2. Materials and Methods

Several laser spectral zone Ascariscopy and Ascaridography systems on a chip were implemented. The Ascariscopes were based on analog matrices - devices with charge-coupled devices (CCD) and output to an oscilloscope-stereogoniometer with X-, Y-, and Z-channels or a standard high-frequency oscilloscope with X-, Y-, and Z-channels. Selective signal output to a multi-channel high-frequency oscilloscope was enabled by a separator with multiple BNC connectors designed by F.A. Nasirov, as shown in the figure above. The digital version (so-called "ascaridographs") was implemented based on CMOS matrices of standard web cameras and CMOS sensors of camera phones. Sample irradiation was carried out in two ways - using incoherent sources (LEDs, the spectral zone of effectiveness of which was selected according to the corresponding type/composition of the semiconductor, as shown in Table 1) and using semiconductor or solid-state lasers, diode-pumped solid-state lasers (DPSS) - as sources of coherent radiation. If in the first case only Schlieren techniques of volume section analysis were possible, in the second case, calculations could be made based on speckle patterns in the projection area of individual anatomical structures. Measurements were carried out synchronously - with visualization in a three-channel scheme in RGB channels (red, green, blue) in the case of using "white" LED and in a sequential mode if the spectrum sources in different subranges were separated (so-called "optical multiplexing"). When using Foveon X3 type matrices, it was possible to measure spectral zone signals at a single point, but in the case of using Bayer filters, only separate ones were used, distributed to the pixel size (of the order of microns) for R, G, and B

	λ (nm)	Semiconductor compounds
	λ > 760	GaAs, AlGaAs
	610 < λ < 760	AlGaAs, GaAsP, AlGaInP, GaP
	590 < λ < 610	GaAsP, AlGaInP, GaP
	570 < λ < 590	GaAsP, AlGaInP, GaP
	500 < λ < 570	InGaN, GaN, GaP, AlGaInP, AlGaP
	450 < λ < 500	ZnSe, InGaN, SiC or Si as a substrate
	400 < λ < 450	InGaN
	λ < 400	BN (λ =215 nm), AlN (λ =210 nm), AlGaN, AlGaInN (< 210 nm)

Table 1: Different LEDs for Different Spectrozonal Regimes of Lens-Less Microscopy

3. Results

The visualization results are presented in a series of figures, for which the decryption is suggested to be done using the cross-sectional scheme in Figure 1:

- When using a red spectral zone diode source, which, unlike the laser with a narrow single wavelength, has a bandwidth of ten to twenty nanometers, not only optically dense contours of stained boundaries of the specimen are visualized well, but also almost all subcuticular structures due to gradient visualization. As a result of the gradient representation, they can be subjected to microphotometric/colorimetric analysis. The brightness level of the non-coherent diode source does not overexpose the central zone of the specimen, thus the "contrast cutoff" effects typical of laser apertureless microscopy are not observed. We conducted a comparative study of visualization capabilities in the R channel for non-coherent and coherent sources, as shown in Figure 1a and Fig. 1b.
- A qualitatively different picture is observed when using the green range - G-channel, as the histological staining of the specimen has significant optical absorption in the green region, resulting in highly pronounced contrast, as in the Schlieren method. Darkened areas are those that were not actively displayed in the red (R) spectral zone channel - for example, the hypodermis and especially the lateral ridges of the hypodermis. Microphotometric analysis at such a gradient and contrast level cannot be performed. However, individual immersion non-uniformities and free, including artifact-free, unstained cellular elements are well visualized, as their refractive index is effective in the green region. This is shown in Figure 1-c.
- To establish the contribution of various pigmented components to the gradient of optical density in the two aforementioned spectral zones, and to select the optimal ascariscopic measurement mode, we conducted a series of experiments to select optimal sources: white/pseudo-white (in additive and subtractive aspects) emission. The principles of measurements on continuum and supercontinuum laser sources are redundant for ascariscopic or more general helminthoscopic tasks from obvious economic perspectives. Therefore, an RGB measurement protocol was adopted with alternately varying combinations of diode sources with different wavelengths.
- The measurement results for the two aforementioned (R+G) channels are shown in Fig. 1d. From the standpoint of refractive and Schlieren methods as a source of histometric da0ta, it can be concluded that the combined use of two spectral zone sources (R, G) in chip-based ascarimetry not only allows for shadow separation and, thereby, determination of specimen volume and height of certain organs above the glass substrate by formulas known from the course of engineering optics but also in a purely analog format (in the absence of channel-based computer processing), provides a spectro-morphological and histochemical picture of the sample, including specimens with multicolored non-fluorescent staining using outdated and rare dyes with localization at a level qualitatively not inferior to fluorescent microscopic (and since Schlieren technique provides volume - then confocal) visualization methods.
- The use of fluorescent, particularly autofluorescent, and chemiluminescent methods necessary for "label-free" analysis can be illustrated as follows: since the spectral ranges and precise excitation wavelengths of fluorescence and emission photon emission are known and well studied, it is possible, knowing the optimal excitation wavelengths as a prerequisite, to analyze non-fluorescent and fluorescent images in colocalization with a small shift relative to these wavelengths. The value of the wavelength deltas may be negligibly small or within a single sub-range and detectable by a single detector (e.g., a separate channel such as R or G or B). Figure 1-e shows a "Schlieren photogram" of a cross-section of an ascarid in the B-channel, performed using a blue diode source ("shortwave spectral zone ascarigram"). Some compartments in this illustration are clearly over-contrasted. However, when shifting further into the shortwave region, in the beam of a violet DPSS laser, the microanatomical details corresponding to these areas generate emission in a longer wavelength/more "red" region, projected not as a shadow but as the glow of the source. This is shown in Figure 1-f - it is quite evident that around 400 nm, many natural and histochemical agents fluoresce well.
- Also it can be obtained positive (Frigure 1-a) and negative (Figure 2-b) visualization of the horse roundworm ovum cell (stained with iron hematoxylin) in the "spectrozonal cube" format, which is a reduced version of the "hyperspectral cube" (the author's unpublished data).

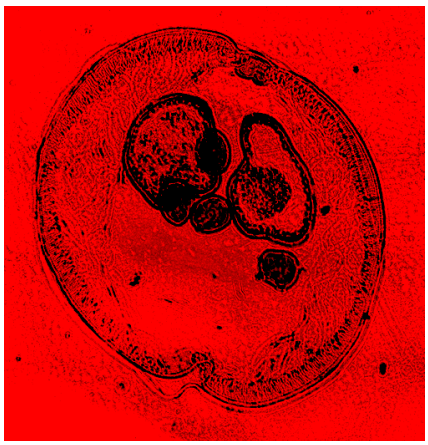

Figure 1a: *Ascaris sp.* Cross-Section. R-Channel Image (LED)

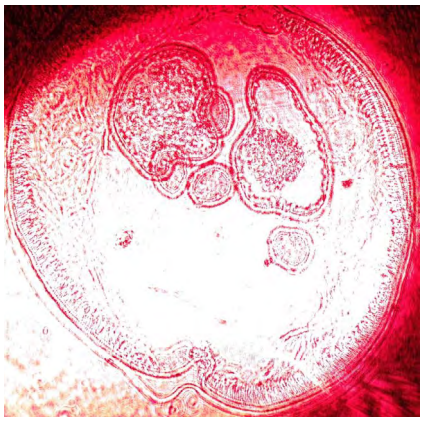

Figure 1b: *Ascaris sp*. Cross-Section. R-Channel Image (Laser Diode)

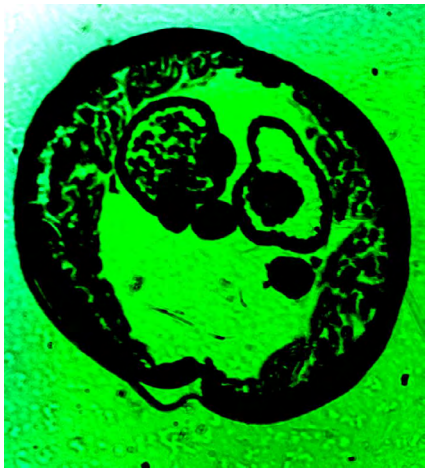

Figure 1c: *Ascaris sp.* Cross-Section. G-Channel Image (LED)

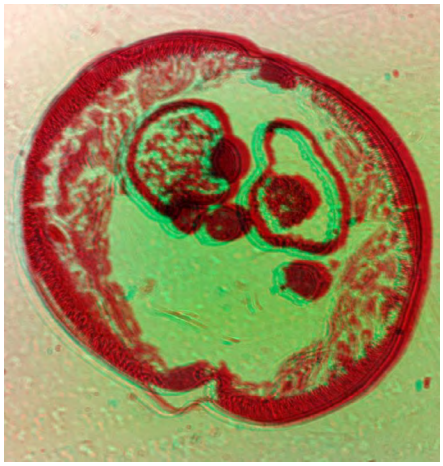

Figure 1d: *Ascaris sp.* Cross-Section. R and G-Channel Shadow Image (LEDs)

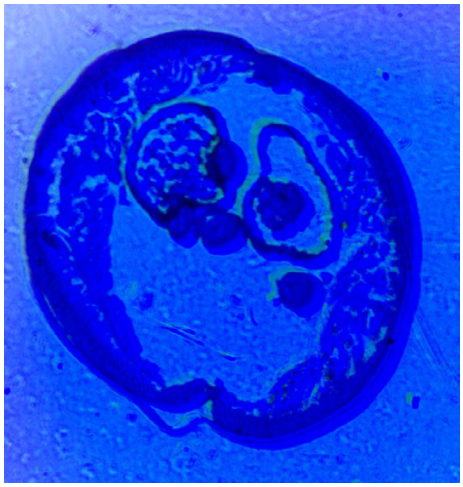

Figure 1e: *Ascaris sp.* Cross-Section. B-Channel Image (LED)

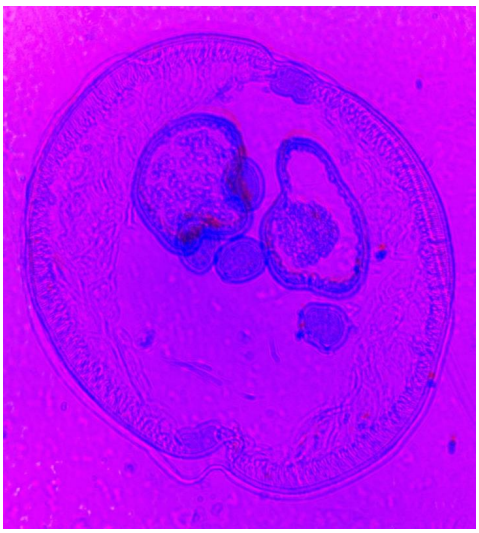

Figure 1f: *Ascaris sp.* Cross-Section. Neat-UV LED

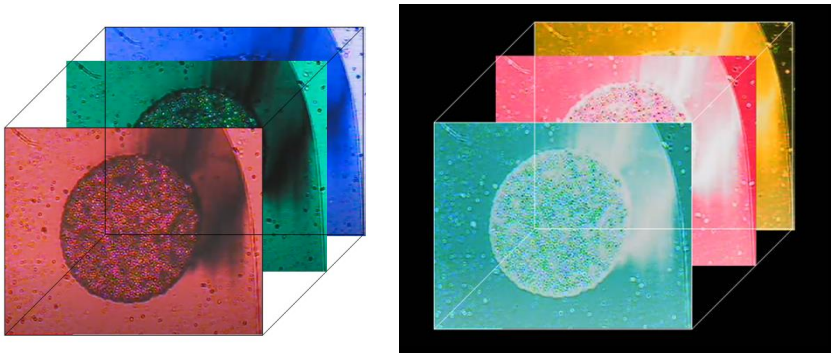

Figure 2: Positive (a) and negative (b) visualization of the horse roundworm ovum cell (stained with iron hematoxylin) in the "spectrozonal cube" format, which is a reduced version of the "hyperspectral cube" (the author's unpublished data).

4. Discussion

A significant challenge for the effective visualization of ascarids is the selection of spectral zone sources, as not all spectral ranges provide precise, high-resolution, and contrast-enhanced depiction of structures, as can be seen from the images presented in the "Results" section. Incomplete spectral sampling does not yield satisfactory results to the extent desired based on resolvometric considerations, or may contain excessive information irrelevant to the measurement of target indicators. The solution to this issue is the subject of one of our upcoming articles, currently available only as a preprint with datasets, scheduled for publication in the journal of this publisher [18]. Some basic development possibilities for the method include:

- Ascarimetry using lasers on metal pairs. This allows for the implementation of laser projection microscopy functions in the device.
- Ascarimetry using terahertz femtosecond lasers. Enables the characterization of chiral structures in tissue with a high degree of reliability.
- Ascarimetry with chemical lasers. Does not require high-voltage power supply, making it implementable in field/polygon conditions.
- Excimer laser ascarimetry. Can be implemented as "theranostics," synchronizing microbeam manipulations on the specimen and property measurements.
- Quantum-laser ascarimetry or laser ascarimetry (LA) with quantum pumping. Initiates thermal processes in the sample, thus can be used as a means of thermoanalytical theranostics.
- IR laser and IR mazer (German: infrarotmaser) ascarimetry. Apart from purely physical aspects, it can be related to the tasks of point 5. Quantum cascade (using semiconductor QCL) ascarimetry also falls under methods of infrared range zooparasitological purpose. Gasdynamic laser ascarimetry with thermal pumping implies only heating by thermal, i.e., infrared source.
- Wavelength-tunable laser ascarimetry using lasers such as titanium-sapphire laser (690—1100 nm), Cr:ZnSe laser (1970—2760 nm), Fe:ZnSe laser (3950-5050 nm), and liquid dye lasers (400—700 nm). The same function can be realized using optical parametric generators in a scheme with laser pumping of a given wavelength, mechatronic crystal orientation changes, thermal regulation of crystal state.

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