Minireview Article

The Use of Immersion Oil in Parasitology Light Microscopic Examination

ABSTRACT

Aims: to briefly review the history of application immersion oil and specifically in some Parasitology Microscopic examination.
Discussion: One way of increasing the optical resolving power of the microscope is to use immersion liquids between the front lens of the objective and the cover slip. The application of immersion oil in microscopic examination began in the mid-17th century. The principle of immersion oil in microscopic examination is to improve the resolution and clarity of the image by reducing the refractive index mismatch between the specimen and the objective lens. In the context of micro-sized parasitic agent, adding this oil will greatly enhanced visualization and in turn will have a direct impact on improving the characteristic morphological image/appearance, thereby enabling observers to optimize microscopic assessment for parasitological examination
Conclusion: The addition of immersion oil in Paarasitology microscopic examination is surely improve optical resolution and clarity of the image and positively support Parasitologist to make correct diagnosis

Keywords: resolution, magnification, lens, numerical aperture object, micro-parasite, protozoan, morphology

1. INTRODUCTION

Light microscopy is a powerful tool for examining micrometric objects (unseen by naked eyes) [1,2], organic (motile or alive) or non-organic (non-motile or in-organic) [3], across a wide range of applications in systems Biology [4] by providing an enlarged image of small structure that deliberately interact with visible light [5], for example their light-absorption, light-reflection and even light-scattering. Light microscopy is used to make small structures and samples visible by using the aid of light, naturally or electrically [1-5]. Contemporary light microscopes are able to magnify objects up to about a thousand times. Since most cells are between 1 and 100 µm in diameter, they can be observed by light microscopy, as can some of the larger subcellular organelles, such as the nucleus, cell membrane, cytoplasm, chloroplasts, mitochondria and cell wall surroundings [6].
This simple microscopic approach is useful to help observer to (1) get the larger picture of the object for identification, (2) analyze and understand the morphologic appearance of the sample for differentiation and even perhaps (3) to explore the material construction in more detail. Light microscope also allows examiners to observe sophisticated scrutiny of the microscopic world, such as how substances diffuse across a cell membrane [1].

Immersion oil is used mainly in high magnification of light microscopic examination to improve the resolution and clarity of the object being observed [7]. Parasitology is very blessed with the existence of microscopic examinations, especially those that use immersion oil, because it helps examiners identify microscopic objects correctly. The aim of this mini review is to reveal the function of immersion oil in Parasitology examination using light microscope and how it works.

2. HISTORY OF IMMERSION OIL

The application immersion oil cannot be separated by the invention of microscope. It is believed that two Dutch spectacle-collaborators and also father-and-son team, Hans and Zacharias Janssen, create the first microscope in 1590; they discovered that, if they intentionally put a lens at the top and bottom of a tube and looked through it, objects observed on the other end of the lens surprisingly became aggrandized [8]. Then in 1667 or 1668, Robert Hooke's well-known presentation, "Lectures and Collections" which was released scientifically in the same year with his masterpiece "Micrographia", which delineates Hooke's extensive analysis using his simple microscope. Hooke was actually the first to suggest the technique of Immersion. He addresses: "that if you would have a microscope with one single refraction, and consequently capable of the greatest clearness and brightness, spread a little of the fluid to be examined on a glass plate, bring this under one of the globules, and then move it gently upward till the fluid touches and adheres to the globule".

By the year 1812, a breakthrough idea of Brewster suggested the application of immersion of the objective into the liquid medium [11] while in almost that same time, Giovanni Battista Amici began pointing the difficult situation regarding chromatic aberration [12] and in 1840, he succeeded making the first working immersion lenses, which initially were designed to be used with oils having the same refraction as glass, homogeneous-immersion [13]. This equipment was far from perfect.

Robert B. Tolles, a genius considered as one among three American Microscope pioneer and builder [14], has a brilliant idea; his insight was increasing the apertures that improves revelation [15]. Amici's main focus was aimed to achieve re-correction from a common condition found in optics named aberrations, a property of optical systems (e.g., lenses), that causes light to be spread out over some region of space rather than focused to a point [16,17].

The principal limitation of the light microscope is its resolving power [5,17-20]. Resolving power of a microscope is a function of refractive index [21-23]. The resolution limit of a microscope is the shortest distance between two nearby objects when the images formed by the microscope are properly differentiated. The resolving power of the microscope is Xmin = 1.22λ/ numerical aperture. Unfortunately, frontiersman microscopists were hindered by three common obstacles, namely (1) optical aberration that unfortunately occur because of lens curvature and dispersion [17], (2) unclear and blurred images due to incorrect use [18], and (3) poor lens design- commonly due to low quality and of imperfect shape, producing sub-optimal imaging [19], which impeded high-resolution inspections until late in the industrialized era [20]. Aberrations were partially able to be corrected by the mid-19th Century, thanks to the introduction of achromatic objectives that reduced chromatic aberration [19] and improvised of numerical apertures [21] to around range from 0.04 for a 1 X objective to 0.95 for a 60 X objective (the largest possible NA) for dry objectives [22] and up to 1.25 for homogeneous immersion objectives for most research grade microscopes condenser lens systems (1000x magnification) [23]. The next section will discuss the

principles and physical properties of immersion oil that commonly use in light microscope that will help augment the size of object being analyzed.

3. IMMERSION OIL: ITS PRINCIPLES AND PHYSICAL PROPERTIES

In light microscopy, oil immersion is a technique used to increase the resolving power of a modern microscope [7]. The application of this oil are aimed to (1) increase the resolution (finer resolution) and (2) improve clarity (brightness) of microscopic image [24]. These two distinctive characteristics are most crucial below high magnification; so it is only the higher power, short focus, objectives that are usually designed for oil immersion [25].

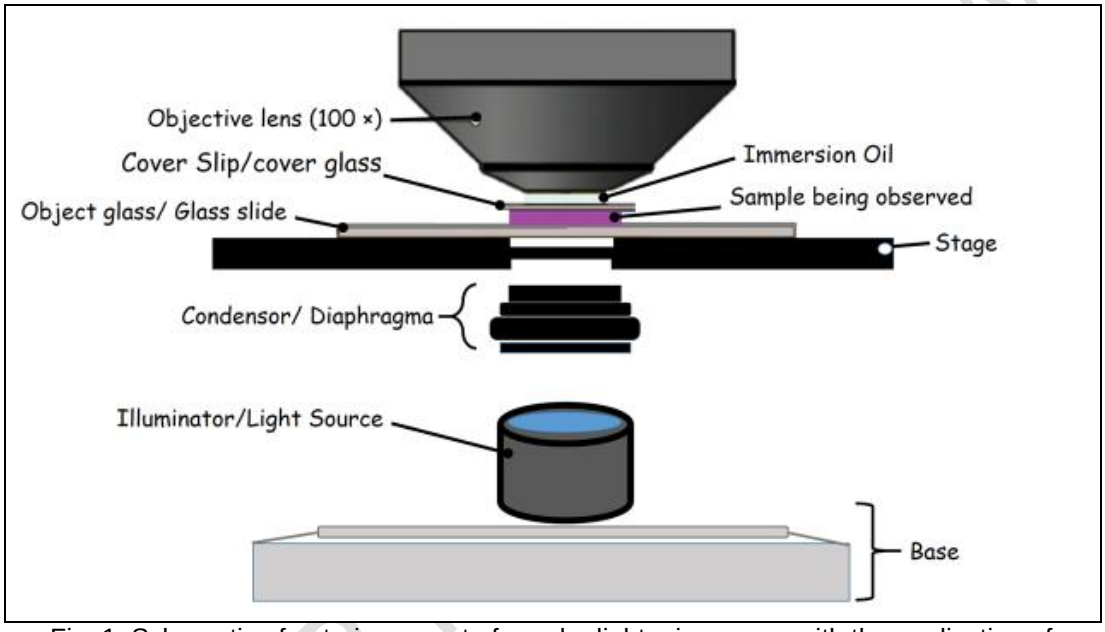

Fig. 1. Schematic of anterior aspect of regular light microscope with the application of immersion oil

The application of this oil is between the front lens of the objective and the cover slip [11,24,25]. Most objectives in the magnification range between 60x and 100x (and higher) are designed for use with immersion oil [26]; and by doing so thereby increasing the numerical aperture of the objective lens. Technically, a gain in resolution by a factor of about 1.518 is attained when immersion oil is substituted for air as the imaging medium. The factor (1.518) which is very close to the refractive index of glass. All reflections on the path from the object to the objective are eliminated in this way.
The oil used must be transparent [24] with high refractive index and It works based on the principle of matching the refractive index between the specimen and the microscope objective lens. By placing a drop of immersion oil between the lens and the specimen, the light rays passing through the specimen are less likely to scatter, resulting in a sharper image [26]. The immersion oil also helps to reduce the loss of light due to refraction at the air-glass interface [25]. This allows for higher magnification and better visualization of fine details in the specimen [11]. The properties of the immersion oil, such as refractive index, viscosity, and erodibility, are important factors in its effectiveness for microscopy [21-23].

The important key of objective lens relies on the beam of light emitted from illuminator that pass through series of media from air into glass (condenser lens) and then into object glass,

specimen being analyzed and then cover slip with its mounting and then immersion oil or air (if no immersion oil used). The direction of the beam is bent and when it passes back from glass to air it is bent back again to its initial, original direction, and this surely impinge with high power lenses because of limited the amount of light which actually can reach and enter the lens, affect the numerical aperture (NA) of the lens and consequently its resolving power. To overcome this bending effect, adding oil to replace the air between the specimen and the lens which has the same refraction index as glass, will surely make the light passes in a straight line from glass through the oil and back to glass as though it were passing through glass all way (see fig. 1 and fig. 2) [27]

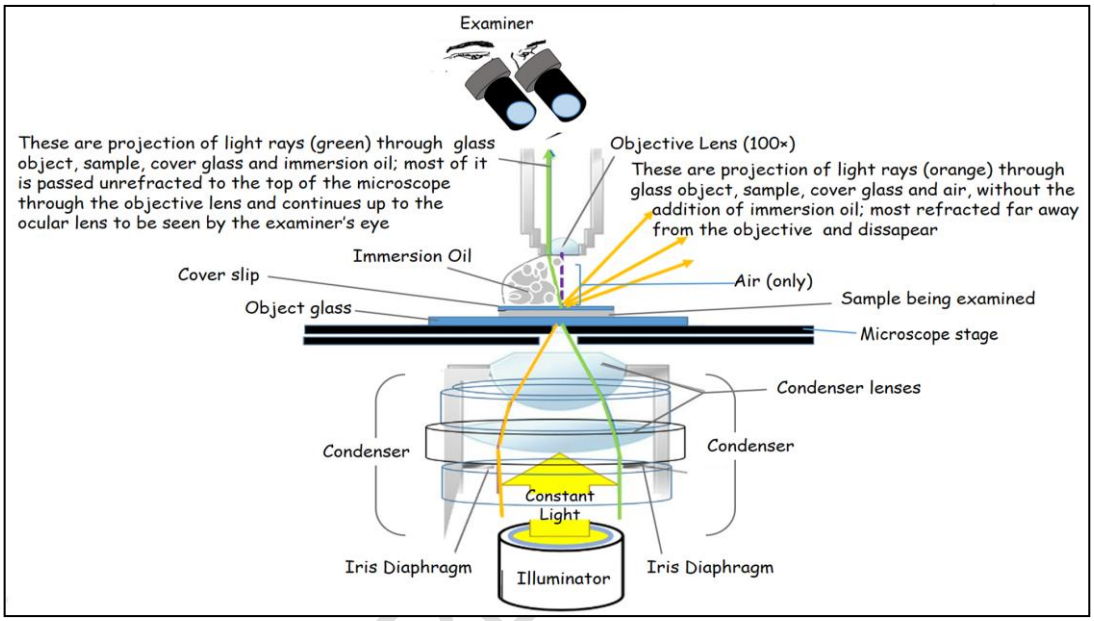

Fig. 2. Schematic representation of the difference of route of refraction, when light pass through immersion oil (left side) and air (right side); objective lens use is 100× magnification. Immersion oil is used in an optical/compound microscope during microscopy analysis in order to boost the resolution of the microscope. It is commonly applied in simultaneity with a high-resolution objective lens (100× magnification) to increase the numerical aperture of the lens, which allows it to gather more light and produce a clearer, higher-resolution image. Immersion oil is placed between the objective lens and the specimen, and it has a refractive index that is very close to that of the glass used in the objective lens. This allows the light to pass through the oil and the glass without being refracted too much, which helps to improve the resolution of the microscope.

Adding Oil immersion in dry lenses actually useless and always changed/deformed the actual images or even distorts it [17]. That is why a complete cleansing of the slide and also the objective lens after using an oil immersion from previous analysis is a must, before using a dry or air objective lens (*i.e.* lens with magnification 5×,10× or 40×) to check or image it again. The use of xylene to remove (clean) immersion oil from microscope slides after examination for acid-fast bacilli have been reported [28]. By Cleaning the entire left over immersion oil from previous application also circumvents tainting the air objective with an improper substance which is not needed [29,30]. So for every microscopists, maintaining the cleanliness of microscope optics is pivotal to ensure the readiness to use and guarantee high-quality imaging [31]. Micro particle such as dust, fingerprints, excess immersion oil, or

mounting medium on or in a microscope causes reduction in contrast and resolution and all of these will directly affecting its imaging performa; especially the lens surfaces which is very fragile and sensitive to scratches, stains and contamination [29-31].

4. APPLICATION OF IMMERSION OIL IN PARASITOLOGY MICROSCOPIC ANALYSIS

If something suspicious is seen by the examiner, a higher magnification may be necessary, and this is the ultimate purpose of using immersion oil in an optical microscope during a stained or wet-mount Parasitology microscopic analysis.

Most parasitic protozoan in humans are small and within range <50 µm in size [32]. The smallest (predominantly as an intracellular forms) are 1-10 µm in diameter or in its longest, but with the exception of *Balantidium coli* may reach up to 150 µm [33]. Protozoa are a polyphyletic group of mono-celled eukaryotes that are actually heterotrophic, self-motile, and lack cell walls [32]. Due to its smaller size, compared to helminths or other type of ectoparasite, protozoan parasites, especially in the intracellular form, present a bigger dispute to make a correct diagnosis based on microscopic examination [34,35].

Globally known small-size disease-causing protozoan parasites include *Plasmodium* spp ranging from 1 to 20 µm in size depend on their stage [36], *Entamoeba histolytica* usually measure range 10 to 15 µm (cyst form) and 20 to 30 µm (cyst form) [37], *Giardia lamblia* whose trophozoites resemble a pear or teardrop and measure 10-20 µm in length, 5-15 µm in width and 1-2 µm thickness [38], *Microsporidia* spp whose resistant spore usually measure 1—4 µm [39], *Cryptosporodium parvum* with its oocysts are measure 4 to 6 µm in diameter [40], *Trichomonas vaginalis* with averages 26 (21-32) µm in total length, with 9.5 (7.4-11.4) µm of body length and 6.8 (5.3-7.7) µm of width [41], *Toxoplasma* spp whose bradyzoites are 7 × 1.5µm [42], *Trypanosoma* spp whose length is about 25 µm in length for *T. brucei* [43], *Babesia* spp with an average size of 4,5 × 2 µm [44], *Leishmania* spp whose amastigotes appear round in shape and 2–4 µm in diameter while cultured promastigotes range between 15 and 25 µm in length and are ellipsoid to slender in shape [45], *Cyclospora cayetanensis* which oocyst very consistent in shape (round or slightly flattened on one side) ans size range only between 8-10 µM [46] and Blastocystis hominis which size varies from 6 to 40 µm [47] .

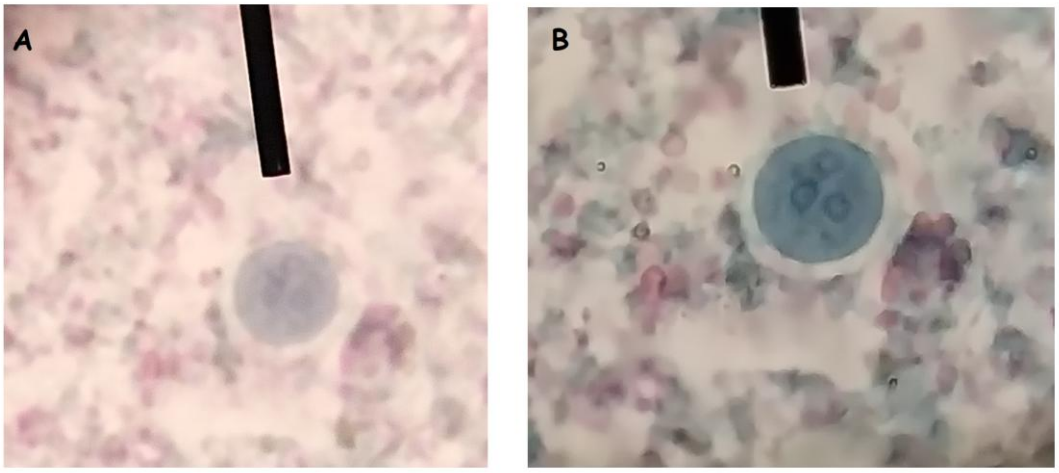

Fig 3. *Entamoeba histolytica*, Cyst form with four nuclei (total magnification 1000× in Olympus CX21LED). A. without Immersion oil, B. with Immersion oil. Photo taken manually using phone cell with both using 3.6 optical zoom. There is a clear difference on clarity and brightness of the image (Slide and microscope courtesy of dept. of Parasitology, faculty of Medicine, Universitas Kristen Indonesia, Jakarta-Indonesia)

Those long lists of previously mentioned small size parasites revealed the complexity of microscopy examination aimed to locate and then to pin point its exact position and followed by magnify the parasite, so that the examiner can confidently determine the typical morphological characteristics, e.g., the species, stage/stadium as well as its density.

5. CONCLUSION

In daily and routine light microscopy use in the laboratory, the goal of adding oil immersion on the sample's cover glass is simply to improve the resolving power of a microscope. This goal is accomplished by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens; all of these means better visualization in size and clarity. In Parasitology, several parasites are actually very small (e.g., *Plasmodium* spp) and in order to visualize them properly, the slide must be analyzing in higher magnification (objective lens 100×) with the addition of a drop of immersion oil.

CONSENT

Not needed

ETHICAL APPROVAL

Not needed

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