The Parasites Caught In-Action: Imaging at the Host–Parasite Interface

Mohammed A. Afifi

1Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, 2Department of Medical Parasitology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

Abstract

For many decades, scientists were unable to expose the invisible existence of the parasites in their living hosts, except by scarification and then dissection of the animal model. This process just demonstrates a dead parasite in a dead host. Using this approach, very limited information can be obtained concerning the dynamics of infection and the pathways utilized by the parasite to survive within a hostile host’s environment. Introduction of ultra-high-speed imaging techniques, with a time domain of barely few microseconds or even less, has revolutionized the “in vivo dissection” of the parasites. Such methods provide platforms for imaging host–parasite interactions at diverse scales, down to the molecular level. These have complementary advantages and relative assets in investigating host–parasite interactions. Therefore, better elucidation of such interaction may require the usage of more than one approach. Precise in vivo quantification, of the parasite load within the host, and better insight into the kinetics of infection are the two main advantages of the novel imaging procedures. However, imaging parasite–host interplay is still a challenging approach due to many constraints related to the parasite biology, the tissue environment within which the parasites exist, and the logistic technical limitations. This review was planned to assist better understanding of how much the new imaging techniques impacted the recent advances in parasite biology, especially the immunobiology of protozoan parasites.

Keywords: Biofluorescence, bioluminescence, imaging, parasites, toxoplasma

INTRODUCTION

A sound recognition and interpretation of the dynamics of host–parasite interplay is crucial for the development of novel therapeutics and vaccines. In vivo dynamics of parasitic infection have remained mysterious. Snapshot images of fixed tissues have proven to be inadequate to make such mechanisms more comprehensible. There is a compelling requirement for novel imaging methodologies to visualize living parasites within living organs and tissues. Imaging parasites in-action is a complicated task and a challenging demand. The parasite–host interplay classically takes place within highly structured, thick, and tightly packed tissue environmental conditions that have constrained the precise discern of such events. Optical microscopy has emerged as one of the most crucial research tools in biomedicine nowadays, and innovated imaging has been of imperative significance. It allowed scientists to look across diverse scales, from full model imaging down to nanoscales. Novel approaches made it possible to look at cell–cell or protein–protein interactions and to visualize molecular events.[1] The widespread availability of novel imaging systems, as well as the establishment of genetically encoded fluorescent peptides, has revolutionized the in vivo dissection of the parasite–host relationship. These innovations have facilitated deep tissue imaging with exceptional resolution and great target specificity. Researchers are now able to achieve real-time monitoring and measurement, of the dynamics of interactions at the host–parasite interface.

Imaging procedures either grant a two-dimensional (2D) description for a specific tissue or a 3D reconstruction model for an organ or a tissue. Novel methods use special probes or modalities to trace definite molecular processes using invasive

Address for correspondence: Prof. Mohammed A. Afifi, Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, P.O. Box. 80205, Jeddah 21589, Saudi Arabia. E-mail: mohafifi36@yahoo.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Afifi MA. The parasites caught in-action: Imaging at the host–Parasite interface. J Microsc Ultrastruct 2021;9:1-6.
or noninvasive approaches of visualization.[2] These approaches have complementary advantages and relative merits in studying host–parasite interactions. Therefore, usage of more than one method or a simultaneous application of a combination of methods is not an uncommon requirement.[3]

**Bioluminescence Imaging**

A molecular imaging approach utilizes luciferase-based gene reporters to perform an in vivo noninvasive imaging in transgenic animals. The light emitted by the enzyme-catalyzed reactions can be applied in visualization of the specific target at cellular and molecular levels. Monitoring at such levels allows reporting the biodynamics and following the fate of cells with high sensitivity. Transgenic, luciferase-expressing cells or animals can be precisely imaged using highly sensitive charge-coupled device cameras, on applying the specific substrate (luciferin).[4] Cellular and molecular processes, such as cell proliferation, differentiation, migration, as well as gene expression and protein–protein interactions, were successfully visualized by bioluminescence.[5] A major disadvantage is the crucial necessity of adequate substrate. This is particularly challenging for long-term monitoring of in vivo dynamics, where continuous supply of substrate would be necessary. Moreover, homogenous substrate distribution depends on the route of administration which could prevent the precise localization of pathogens.[6]

The dynamics of eukaryotic infections by protozoan parasites such as *Plasmodium, Toxoplasma, Leishmania*, and *Trypanosoma* have all been observed using bioluminescence imaging (BLI). Sequestration patterns of the schizont stage can be analyzed within 1–2 days after infection by studying *Plasmodium berghei*, in a rodent model, using firefly luciferase (FLuc) under schizont-specific promoter ama1.[7] In vivo BLI has also proved superior, compared to quantitative reverse-transcription polymerase chain reaction, for real-time monitoring of the kinetics of pre-erythrocytic malaria in a murine model infected with *Plasmodium yoelii*. The same study reported a positive evaluation of the in vivo BLI screening of antimalarial efficacy.[8]

Real-time monitoring of the kinetics of *Toxoplasma gondii* infection in a mouse model revealed a direct relationship between emitted photon levels and the parasite burden, allowing the quantification of the parasite load in vivo.[9] Another noninvasive BLI study was able to demonstrate toxoplasma dissemination in a murine model. The competency of BLI for successful monitoring of toxoplasma reactivation as well as the efficacy of anti-toxoplasma chemotherapy was also displayed.[10]

The development of a double-transfected murine model of visceral leishmaniasis, with avirulent strain of *Leishmania donovani*, expressing two reporter genes – bioluminescent (FLuc) and fluorescent (E2-crimson) – enabled an in vivo real-time monitoring of the infection dynamics in visceral leishmaniasis. It also facilitated the quantification of the parasite load in each organ. This bioluminescence model could allow for long-lasting follow-up studies, with easy and precise day-by-day visualization of the infection status.[11]

**Biofluorescence Imaging**

This is an imaging technology that depends on utilizing fluorescent-labeled peptides or dyes for precise visualization of pathogen’s niches inside the host as well as the dynamics of pathogen–host interplay at cellular and molecular levels.

Typical fluorescence imaging relies on a linear interaction between light and matter where if a single photon of light reacts with a fluorescent molecule; it will emit only one fluorescent photon. However, multiphoton imaging, especially the two-photon excitation microscopy, which depends on nonlinear interactions and complex quantum mechanical effects, is the technique of choice for biofluorescence imaging.[12]

**Two-Photon Microscopy and Analytical Dynamics of Parasite–Host Interplay**

Little was known about the maneuvers used by parasites to combat or evade the ongoing mechanisms adopted by the host to clear such intruders. The emergence of genetically-encoded fluorescent-labeled reporters as well as the development of the two-photon microscopy has made it feasible to monitor the kinetics of infection within living tissues.[13] Multiphoton microscopy has provided the chance to accomplish a long sought-after goal, real-time imaging of the dynamic interplay between the parasite and host–cell. Multiphoton microscopy utilizes low-energy photons in short pulses to perform imaging at previously unreachable depths within tissue with reduced light scattering and minimal photobleaching.[14] It is possible to lively trace the detailed events of an immune reaction and closely monitor the kinetics of specific cells (e.g., T-cells), while interacting with parasites in a living tissue environment. This approach has been principally redefined our concepts toward the exact roles and functions of the immune cells against invading pathogens mainly through enhancing the examination of immune reactions to representative antigens in lymph nodes.[15,16]

**Two-Photon Microscopy and Intracellular Parasites**

Intracellular protozoans are causative agents for significantly serious parasitic diseases. Millions are infected annually with substantial morbidity, mortality, and economic loss.[17] Some of these protozoans, such as *Plasmodium, Leishmania*, and *Toxoplasma*, typically initiate a chronic intracellular existence justifying their abilities in manipulating host immune defenses and hijacking its metabolic machinery for survival, proliferation, and transmission. The intracellular niche also confers a shield from host’s immunity.[18] While an evident heterogeneity of pathogenetic pathways is employed by such parasites, a clear consensus exists regarding the signaling
hubs orchestrated by the parasites. Therefore, dissection of
the events of host–parasite interplay at all levels from system
to molecule is crucial to differentiate between potentially fatal
from uncomplicated infections.

Some of the unique advantages of two-photon microscopy
make it a suitable approach to study the dynamics of
parasitic infections, particularly those caused by intracellular
protozoans. First, two-photon microscopy allows better
and real-time visualization of fluorescent-labeled parasites
and immune cells, as they interact. Second, two-photon
microscopy has improved the in vivo visualization of
parasite–host interplay in complex tissue environments.[19]
Finally, two-photon approaches are constantly advancing and
allow live-imaging parasite dynamics in thick tissues that have
never accessed before. These unique advantages facilitated
the study of cell signaling, real-time cytokine production,
distinguishing between invasion and phagocytosis, identifying
cells manipulated by intracellular parasites, and tracking the
kinetics of infection and the dissemination of the parasite
through photo-convert and track invaded cells.[20–22]

Considering the multiplicity of infectious behaviors of different
intracellular protozoans, this section focuses only on the
mechanisms utilized by T. gondii to manipulate and hijack
host’s cellular and molecular machinery. It also demonstrates
how live-imaging by two-photon microscopy has advanced
our understanding of such mechanisms.

Two-Photon Microscopy and Space/Time
Characterization of Toxoplasma gondii Infection

A complex relationship does exist between T. gondii and its
human host. Infection with T. gondii has two distinct phases,
regarding the developmental parasitic stage as well as the
niches of parasite existence. In the acute phase, infection
starts in the intestine; however, Toxoplasma tachyzoites soon
use the host immune cells as migratory vehicles to spread
through the body and cross several unique anatomical and
cellular barriers such as the blood–brain barrier, blood–retina
barrier, and mother–fetus placental interface. The replication
of parasites, at these sites, results in the most serious clinical
presentations which are encephalitis, chorioretinitis, and
congenital toxoplasmosis, respectively.[23,24] Because of a
robust immune response, tachyzoites transform into the slow-replicating bradyzoites that are packed in tissue cysts
that characterize the chronic phase and predominantly exist in
the nervous tissues as well as muscles. Throughout such stage,
T-cells and interferon-γ are crucial to maintain chronicity and
inhibit recrudescence, leading to persistence of infection for
the lifetime of the host.[25]

No therapeutics are readily available to kill the cyst, the
hallmark of chronic toxoplasmosis. This is mainly because of
the deficient monitoring of the host–parasite interplay
and the progression events of toxoplasma infection. Tracing
the host–parasite interactions throughout acute as well as
chronic phases of the disease may result in developing sound
approaches for future therapeutics.[26] The events that take place
throughout such dynamic host–toxoplasma interactions are
complicated and difficult to monitor in vivo. A comprehensive
recognition of these events requires an efficient tracking, within
a milieu of multiplex tissue environments, of toxoplasma,
its secretory and excretory products, as well as incriminated
immune cells. This can be significantly achieved by direct
imaging of toxoplasma and immune cells within an in vivo
model.[27]

Two-Photon Imaging and Toxoplasma Dynamics in
the Intestine

Apart from congenital transmission, toxoplasmosis in humans
is mostly acquired through the oral route. Ingestion of the
sporulated stage of the oocyst passing in cat’s feces or the tissue
cyst in undercooked meat of the many animal intermediate
hosts is the main route of infection.[24] Therefore, intestine is
usually the first station in the long journey of T. gondii in
the human body. After oral infection, toxoplasma starts to
replicate within intestinal epithelium and then initiates a rapid
dissemination throughout the body. The exact kinetics of this
intestinal phase and the triggering of an immune reaction were
poorly understood. Visualization of the events, during acute
toxoplasma infection, has achieved better understanding of the
essence of a protective immune response against toxoplasma
during the intestinal phase. Two-photon imaging was able to
elucidate the kinetics of spread and colonization of toxoplasma
tachyzoites, in the intestines of orally infected murine model.[28]
Very early during infection, T. gondii parasites scatter among
the villi with a frequent pattern of distribution of one villous
containing one parasite. Few days later, foci of infection have
developed showing clusters of villi packed with parasites that
tend to settle toward the tips of the villi.[29] Another interesting
point cleared by live-imaging is the phenomenon of hasty
relocation of toxoplasma-infected dendritic cells (DCs) far
from the intestine. T. gondii infects intestinal CD11c+ DCs
within 3 days after infection and is thought to take these cells
as vehicles to translocate from the intestine to the lymph
nodes.[30] Live-imaging 6–8 days after oral toxoplasma
infection of CD11c-YFP reporter mice has demonstrated that
heavily infected villi became dramatically devoid of
CD11c-YFP+ cells, in contrast to the neighboring uninfected
villi that retained a dense existence of CD11c-YFP+ cells.[31]
recruitment to the lymph node in reaction to toxoplasma infection.[23] The in vivo multiphoton microscopy studies[23,34] revealed characteristic transient and persistent dynamic swarms of neutrophils, coinciding with macrophage clearance from the subcapsular spaces. The “swarms” are signaled by toxoplasma exit from cells and/or invited migration of some “pioneer” neutrophils.[31] As mentioned earlier, DC another innate immunity cell type is essential to develop resistance to acute toxoplasma infection.[28,35] DCs are well-recognized providers of interleukin-12 in early toxoplasma infection, initiating defensive T-cell responses very early during infection.[36] Multiphoton imaging demonstrates that toxoplasma-infected DCs are hypermotile which facilitate dissemination of the parasite.[37] Early after infection (first 48 h), the antigen-primed T-cells and DCs were involved in an intimate cross-talk,[32,34] within the lymph node. An active handover of toxoplasma was observed, from parasitized DCs to primed T-cells.

The characteristic “neutrophil swarms” caught by multiphoton microscopy coincide with lymph node tissue remodeling, especially the CD169+ macrophage layer. This results in “gaps” that permitted other immune cells such as natural killer (NK) cells to have access.[33] The lymph node remodeling predominantly took place in the subcapsular region and results in toxoplasmatriggered accumulation of NK cells in the remodeled regions.[38] Twophoton imaging showed a striking proliferation of collagen fibers in toxoplasmainfected lymph nodes.[34] This proliferation allows better interaction between collagen and NK cells and results in better activation of such immune cells with subsequent control of infection.[33]

**Two-Photon Imaging and Toxoplasma gondii in the Brain: A Partially Revealed Layout**

On reaching the brain, *T. gondii* invades microglia and astrocytes, before transforming into cysts mainly within neurons. A continuing adaptive immune response critically orchestrated by CD8 T-cells.[19,40] Immune responses to toxoplasma are tailored to control the chronic phase of toxoplasma infection in the brain as well as to limit the damage to host tissues. This balance is exceptionally crucial in the brain, which is a vital, delicate tissue that exhibits a limited power of regeneration.[41] Therefore, immune cell migration to the brain is largely controlled, and the actions of the recruited ones are strictly checked. Multiphoton technology revealed unprecedented details of toxoplasma and brain cell behaviors during chronic infection. It has offered significant insights into the kinetics of the interplay between toxoplasma and brain immune cells, explaining how this balance is sustained.

Two-photon microscopy-based study showed that antigen-primed CD8+ T-cells migrated to the brain tissues of chronically-infected murine models and remained there as long as antigen existed.[42] Another study revealed clustering and arrest of T-cell populations near infected brain cells as well as remodeling and upregulation of the brain fibrous network, like what is seen in the lymph nodes. It was also noted that CD8 T-cells migrated to areas of toxoplasma replication along this fibrous network. The same study, further, demonstrated the already recognized phenomenon of astrocyte activation during chronic toxoplasmosis,[43] including astrocytic swelling.[44] Two-photon technology has demonstrated a novel viewpoint of CD8 reactions to chronic toxoplasma brain infection. It has revealed an exceptional mechanism of Antigen (Ag) recognition where T-cells overlook Ag-bearing brain cysts, while adopting transient contacts with antigen-presenting cells (APCs) and granuloma-like foci may close to areas of toxoplasma dissemination. This manner of interplay most probably indicates immune evasion by toxoplasma. It could also reflect the ability of the local brain environment to restrict Ag presentation and modulate immune reaction. This mode is close to that done by CD8 T-cells where they act as source of cytokines to APCs rather than commit direct killing of target cells.[42]

Thus, we can deduce that dynamic multiphoton imaging has allowed unprecedented insight into the dynamics of toxoplasma infection, explaining (i) how specific immune responses, against the parasite, are dynamically orchestrated in different local tissue environments, (ii) how defensive reactions to toxoplasma are elicited and controlled, and (iii) how the parasite evades such immune mechanisms to survive inside the host.[31]

The fundamental advantages of two-photon microscopy are better 3D and deep tissue imaging with reduced phototoxicity and photobleaching. However, it does not achieve an enhancement of spatial resolution with a high possibility of photodamage, especially for visible fluorophores. Therefore, weighing the advantages and limitations, in each situation, is crucial to determine when the two-photon microscope is appropriate to use.[45]

**Confocal Imaging**

This is an optical technology that is particularly useful for 3D imaging. It provides an image sectioning potential to obtain 3D imaging data from specimens with diverse depths by differentiating between in-focus signals from the disturbing background flaw.[46] Confocal microscopy assembles light from a diffraction-limited focused spot (<1 μm in thickness) in the specimen. A pinhole is assigned to track the path and cast-off the out-of-focus light to enhance resolution and contrast. The light source, the detection pinholes, and the in-focus specimen spot are collectively called “con-focal.”[42]

Numerous models of confocal microscopy are accessible. The one that is commonly used is laser-scanning confocal microscope, which seizes images through specimen scanning with a compact laser beam, then assembling the emitted signals using a photodetector.[47] Confocal microscopy is usually applied in imaging the interaction of microorganisms within their hosts,[48] including microbial adhesion and invasion,[49] intracellular motility,[50] and the kinetics of the interplay between toxoplasma and brain immune cells, explaining how this balance is sustained.
and immune response to infection.\cite{51} It was used for direct imaging the motility of living malaria parasites within the salivary glands of infected mosquito vectors.\cite{52} A high-speed laser spinning disk confocal imaging system allowed an in vivo 4D imaging of malaria parasite vector–host transmission.\cite{53,54} Imaging showed motile sporozoites gliding away from the bite site and targeting capillary blood vessels. It then showed sporozoite penetration of capillary endothelium as well as its entrance to the capillary lumen to flow in the blood stream, presumably heading toward the liver.\cite{55}

Confocal microscopy was also used to measure glutathione (GSH), a key player in the redox mechanisms in malaria parasites. Parasite’s GSH combats the oxidative stress that faces the parasite once internalized within the host red blood cells. Reactive oxygen and nitrogen species are also produced by the host’s immune system, as a product of hemoglobin digestion, and by antimalarial drugs.\cite{56} GSH is usually measured with conventional methods, such as reverse-phase high-performance liquid chromatography or glutathione reductase-dependent reactions, which cannot achieve a dynamic thiol measurement.\cite{57} Real-time imaging of the potential of glutathione redox in the obligatory intracellular Plasmodium falciparum was performed using a glutathione biosensor consisting of human glutaredoxin-1 linked to a green fluorescent peptide. Confocal microscopy was able to demonstrate the rapid changes in GSH levels as a reaction to the host oxidative and nitrosative stress. This technique was also able to detect thiol changes induced by oxidative actions of some antimalarial drugs.\cite{58}

Confocal microscopy, however, has its limitations. While it allows the capture of high-resolution 3D images for cells in cultures or thin specimens, its capability to image thick tissues is limited. Confocal technology necessitates a point-by-point scan in the lateral and axial directions to facilitate 3D reconstructive images of the assigned tissue. In addition, axial scanning in confocal is slower than scanning in lateral directions.\cite{59} There is also a poor confocal imaging on focusing deeper in tissues due to rapid decay of intensity and contrast as the number of emitted photons, arising from the scanned sample, decays exponentially with the increasing depth. Confocal technique is particularly vulnerable to photon scattering. The dispersed photons not only unable to gather in an assigned focus, but they also contribute in diffuse background and consequently photobleaching. Thus, confocal microscopy has a major flaw that renders it impractical in samples of <100-μm thickness where only 1% of the emitted fluorescence contributes to confocal imaging.\cite{2}

Therefore, good resolution is practically confined to depths of 60–80 μm.\cite{59} One of the inherent drawbacks of confocal microscopy is that it needs ultraviolet (UV) excitation. Whenever UV emission is needed to capture specific signals from live specimens, we must trade out imaging resolution with sample integrity. Photons emitted from UV excitation exhibit excessive energy, and therefore, cell damage and/or photobleaching are expected on using high intensity.\cite{12}

**CONCLUSION**

Innovative microscopic approaches, together with the emergence of smart genetically-encoded bioluminescent and/or biofluorescent probes, have consistently improved our tools for real-time monitoring of pathogens and immune cells. Our understanding of the host–parasite interaction has transformed from just descriptive 2D information to precise 3D and 4D in vivo checking of such interactions down to the cellular as well as the molecular levels. This has significantly improved our understanding of how the host–parasite dialog proceeds in complex tissue environments. We described herein examples of advances in microscopy that contributed to upgrade the concepts related to host–parasite interactions of some intracellular parasites. We are confident that with the widespread usage of such modern techniques as well as the introduction of the next-generation imaging approaches, such as light sheet fluorescence microscopy, label-free 3D imaging by holotomography microscopy, and superresolution microscopy, specifically single-molecule localization microscopy will lead to update the fundamental knowledge on the immune responses as well as the pathophysiology of parasitic infections. This might create new platforms for drug discovery and vaccine development.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Millington OR, Myburgh E, Mottram JC, Alexander J. Imaging of the host/parasite interplay in cutaneous leishmaniasis. Exp Parasitol 2010;126:310-7.
2. Oheim M, Michael DJ, Geisbauer M, Madsen D, Chow RH. Principles of two-photon excitation fluorescence microscopy and other nonlinear imaging approaches. Adv Drug Deliv Rev 2006;58:788-808.
3. de Niz M, Spadin F, Marti M, Stein JV, Frenz M, Frischknecht F. Toolbox for in vivo imaging of host-parasite interactions at multiple scales. Trends Parasitol 2019;35:193-212.
4. Mezzanotte L, van’t Root M, Karatas H, Goun EA, Löwik CW. In vivo molecular bioluminescence imaging: New tools and applications. Trends Biotechnol 2017;35:640-52.
5. Avci P, Karimi M, Sadasivam M, Antunes-Melo WC, Carrasco E, Hamblin MR. In vivo monitoring of infectious diseases in living animals using bioluminescence imaging. Virulence 2018;9:28-63.
6. Claes F, Vodnala SK, van Reet N, Boucher N, Lunden-Michel H, Baltz T, et al. Bioluminescent imaging of Trypanosoma brucei shows preferential testis dissemination which may hamper drug efficacy in sleeping sickness. PLoS Negl Trop Dis 2009;3:e486.
7. Franke-Fayard B, Waters AP, Janse CJ. Real-time in vivo imaging of transgenic bioluminescent blood stages of rodent malaria parasites in mice. Nat Protoc 2006;1:476-85.
8. Miller JL, Murray S, Vaughan AM, Harupa A, Sack B, Baldwin M, et al. Quantitative bioluminescent imaging of pre-erythrocytic malaria parasite infection using luciferase-expressing Plasmodium yoelii. PLoS One 2013;8:e60820.
9. Subauste C. Animal models for Toxoplasma gondii infection. Curr Protoc Immunol 2012;96:1-23.
10. Saeij JP, Boyle JP, Grigg ME, Arrizabalaga G, Boothroyd JC. Bioluminescence imaging of Toxoplasma gondii infection in living mice reveals dramatic differences between strains. Infect Immun
2005;73:695-702.
11. Melo GD, Goyard S, Lecoeur H, Rouault E, Pescher P, Fiette L, et al. New insights into experimental visceral leishmaniasis: Real-time in vivo imaging of Leishmania donovani virulence. PLoS Negl Trop Dis 2017;11:e0005924.
12. Ustione A, Piston DW. A simple introduction to multiphoton microscopy. J Microsc 2011;243:221-6.
13. Yuste R. Fluorescence microscopy today. Nat Methods 2005;2:902-4.
14. McGovern KE, Wilson EH. Dark side illuminated: Imaging of Toxoplasma gondii through the decades. Parasit Vectors 2013;6:334.
15. Sumen C, Menpel TR, Mazo IB, von Andrian UH. Intravital microscopy: Visualizing immunity in context. Immunity 2004;21:315-29.
16. Cahalan MD, Parker I. Choreography of cell motility and interaction dynamics imaged by two-photon microscopy in lymphoid organs. Annu Rev Immunol 2008;26:585-626.
17. Mahanta A, Ganguli P, Barah P, Sarkar RR, Sarmah N, Phukan S, et al. Integrative approaches to understand the mastery in manipulation of host cytokine networks by protozoan parasites with emphasis on Plasmodium and Leishmania species. Front Immunol 2018;9:296.
18. Sacks D, Sher A. Evasion of innate immunity by parasitic protozoa. Nat Immunol 2002;3:1041-7.
19. Coombs JL, Robey EA. Dynamic imaging of host-pathogen interactions in vivo. Nat Rev Immunol 2010;10:353-64.
20. Ch탄anov T, Hampton HR, Waterhouse LA, Wood K, Tomura M, Miwa Y, et al. Real-time interactive two-photon photomorphogenesis of circulating lymphocytes for discontinuous cell tracking in live adult mice. J Biophotonics 2014;7:425-33.
21. Garrod KR, Moreau HD, Garcia Z, Lemaître F, Bouvier I, Albert ML, et al. Dissecting T cell contraction in vivo using a genetically encoded reporter of apoptosis. Cell Rep 2012;2:1438-47.
22. Benucci H, Lemaître F, Deguine J, Moreau HD, Bouvier I, Garcia Z, et al. Visualizing the functional diversification of CD8+ T cell responses in lymph nodes. Immunity 2010;33:412-23.
23. Barragan A, Hitziger N. Transepithelial migration by Toxoplasma. Subcell Biochem 2008;47:198-207.
24. Montoya JG, Lienfendfeld O. Toxoplasmosis. Lancet 2004;363:1965-76.
25. Suzuki Y. Host resistance in the brain against Toxoplasma gondii. J Infect Dis 2002;185 Suppl 1:S58-65.
26. Pittman KJ, Knoll LJ. Long-term relationships: The complicated interplay between the host and the developmental stages of Toxoplasma gondii during acute and chronic infections. Microbiol Mol Biol Rev 2015;79:387-401.
27. John B, Weninger W, Hunter CA. Advances in imaging the innate and adaptive immune response to Toxoplasma gondii. Future Microbiol 2010;5:1321-8.
28. Coombs JL, Charsar BA, Han SJ, Ch탄anov T, van Dooren GG, Herzmark P, Chen Y, et al. Dynamic imaging of T cell-parasite interactions in the brains of mice chronically infected with Toxoplasma gondii. J Immunol 2009;182:6379-93.
29. Fischer HG, Nitzen G, Reichmann G, Hadding U. Cytokine responses induced by Toxoplasma gondii in astrocytes and microglial cells. Eur J Immunol 1997;27:1539-48.
30. Wilson EH, Harris TH, Måss P, John B, Tait ED, Wu GF, et al. Behavior of parasite-specific effector CD8+T cells in the brain and visualization of a kinesis-associated system of reticular fibers. Immunity 2009;30:300-11.
31. Bennenger RK, Piston DW. Two-photon excitation microscopy for the study of living cells and tissues. Curr Protoc Cell Biol 2013;59:1-24.
32. Wang PH, Singh VR, Wong JM, Sung KB, Luo Y. Non-axial-scanning multifocal confocal microscopy with multiplexed volume holographic gratings. Opt Lett 2017;42:346-9.
33. Smith CL. Basic confocal microscopy. Curr Protoc Neurosci 2011;Chapter 2:Unit 2.2.
34. Roux P, Münter S, Frischknecht F, Herbomel P, Shorte SL. Focusing light on infection in four dimensions. Cell Microbiol 2004;6:333-43.
35. Elphick GF, Querbes W, Jordan JA, Gee GV, Eash S, Manley K, et al. The human polymavirus, JCV, uses serotonin receptors to infect cells. Science 2004;306:1380-3.
36. Vlachou D, Zimmermann T, Cantera R, Janse CJ, Waters AP, Kafatos FC. Real-time, in vivo analysis of malaria ookinete locomotion and mosquito midgut digestion. Cell Microbiol 2004;6:671-85.
37. Perrin AJ, Xiang X, Birmingham CL, So NS, Brumell JH. Recognition of bacteria in the cytosol of Mammalian cells by the ubiquitin system. Curr Biol 2004;14:806-11.
38. Frischknecht F, Baldacci P, Martin B, Zimmer C, Thibere S, Olivo-Marin JC, et al. Imaging movement of malaria parasites during transmission by Anopheles mosquitoes. Cell Microbiol 2004;6:687-94.
39. Amino R, Thibere S, Martin B, Celli S, Shorte S, Frischknecht F, et al. Quantitative imaging of Plasmodium transmission from mosquito to mammal. Nat Med 2006;12:220-4.
40. Thibere S, Blazquez S, Baldacci P, Renaud O, Shorte S, Ménard R, et al. In vivo imaging of malaria parasites in the murine liver. Nat Protoc 2007;2:1811-8.
41. Aulner N, Danckaert A, Fernandes J, Nicola MA, Roux P, Salles A, et al. Fluorescence imaging host pathogen interactions: Fifteen years of benefit of hindsight….Curr Opin Microbiol 2018;43:193-8.
42. Bozdzech Z, Ginsburg H. Antioxidant defense in Plasmodium falciparum – Data mining of the transcriptome. Malar J 2004;3:23.
43. Morgan B, Sobotta MC, Dick TP. Measuring E(GSH) and H2O2 with roGFP2-based redox probes. Free Radic Biol Med 2011;51:1943-51.
44. Katsou D, Mohring F, Rahls S, Meyer AJ, Becker K. Real-time imaging of the intracellular glutathione redox potential in the malaria parasite Plasmodium falciparum. PLoS Pathog 2013;9:e1003782.
45. Dmitriev RI, Borisov SM, Düssmann H, Sun S, Müller BJ, Prehn J, et al. Versatile conjugated polymer nanoparticles for high-resolution O2 imaging in cells and 3D tissue models. ACS Nano 2015;9:5275-88.