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The Physical Principles of Light Propagation and Light-Matter Interactions

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What You Will Learn in This Chapter

This chapter outlines a basic and introductory understanding of the light, its behavior, and its interaction with matter. A definition of light is noted, as well as a description of the particle and wave properties, and the polarization, refraction, reflection, diffraction, and absorption of light. The diffraction limit and optical light filtering have been discussed with respect to microscopy. This chapter provides an introduction education into the underlining fundamental physics to which all microscopy is built upon.

1.1 Definitions of Light

Light exhibits properties of both waves and of particles. This is known as waveparticle duality and states the concepts that every quantum entity can be described either as a particle or as a wave [1].

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When dealing with light as a wave, wavelengths are assigned to describe the single wave cycle. The entire electromagnetic spectrum, from radio waves to gamma rays, is defined as "light." Yet often when discussed, people generalize light to identify just the visible spectrum, i.e., the light accepted by human vision. Visible light has wavelengths in the range of 380-740 nm (1 nm equals 10^{-9} m). Surrounding the visible spectrum is ultraviolet radiation, which has shorter waves, and infrared radiation, which has longer waves. For microscopy purposes, the ultraviolet, visible, and infrared spectrums provide a vital role. Naturally, light is never exclusively a single wavelength, but rather a collection of waves with different wavelengths. In the visible spectrum, the collection of waves manifests as white light, with each wavelength providing a unique color from violet to red.

When characterizing light by wavelength, it is often intended within a vacuum state, as vacuum has a unity refractive index. When traveling through a vacuum, light remains at a fixed and exact velocity of 299,792,458 m/s, regardless of the wavelength and movement from the source of radiation, relative to the observer. The velocity is usually labeled c, which is descriptive for the Latin word "celeritas," meaning "speed." Wavelengths are designated with the Greek letter lambda, λ .

Light, as previously mentioned, is the electromagnetic spectrum consisting of electromagnetic waves with variation in the one wave cycle distance, and as the name implies are synchronized with oscillating electric and magnetic fields. Another property of light that is often referred to is the frequency. This describes the number of periods per time unit the electric field waves oscillate from one maximum to another maximum. Frequency is conventionally designated *f*, and within vacuum follows the relation of $c = \lambda f$. As light passes through matter (*anything including air but excluding a vacuum*), the velocity and wavelength decrease proportionally. Occasionally the wavenumber, I/λ , can be found to characterize light and is symbolized by $\overline{\nu}$ with a common unit of cm⁻¹.

When referring to light as a particle, namely a photon, it is assigned an amount of energy, *E*. The energy is intrinsically connected to the wave properties of light by the momentum of the photon and its wavelength, or inversely, its frequency; $E = hv = hc/\lambda$. Where *h* is Planck's constant with the value 6.626,070,150 × 10⁻³⁴ Joules per second.

1.2 Particle and Wave Properties of Light

Previously, I mentioned the duality nature of light and described them independently of one another. This seems a strange concept that light can exhibit properties of both waves and of particles. The notion of duality is entrenched in a debate over the nature of light and matter dating back to the 1600s, where Huygens and Newton proposed conflicting theories of light. The work by Schrödinger, Einstein, de Broglie, and many others has now recognized that light and matter have both wave and particle nature and is currently an appropriate interpretation of quantum mechanics [2].

One such experiment that demonstrates this strange duality behavior is the Young's double slit experiment [3]. The American physicist and Nobel prize winner

Richard Feynman said that this experiment is the central mystery of quantum mechanics. To explain really how bizarre this concept is, I will try to describe the experiment.

Imagine that there is a source of light shining against a screen with two slits. Importantly, this source of light must be monochromatic light (*light of a particular wavelength or color*). The light exits the source in waves like ripples in a rain puddle, that is the nature of wave-like behavior, and as the light hits the screen and exits through the two slits, it allows each individual slit to act almost like a new source of light. As such, the light extends out through the process of diffraction, and as the waves of these two "new" light sources overlap they interfere with one another. This creates crests and troughs within the diffraction pattern, such that when a crest hits a trough, the light cancels, and where a crest hits a crest, they will amplify. This results in an interference pattern displaying on the back screen a series of light and dark fringes when the light waves have either canceled or amplified in phase. This in itself is a rather simple process to understand and this wave-like property has been known since the early nineteenth century.

Imagine conducting the same experiment again, but rather than using waves, instead let us consider particles. Envisage pouring grains of sand through two slits, rather than waves propagating out and constricting through the individual slits. Each particle of sand would either go through one slit or the other, and the output imaginable would be two mounds of sand underneath each slit. Therefore, two distinct peaks are reminiscent of particle-like behavior, whereas a multiple peaks pattern is a wave-like behavior. However, what really happens when the sand particles are replaced with photons, particles of light? Let us first consider blocking of a single slit and project a stream of photons through the single open slit. Nothing particularly strange happens here, and the profile displayed on the back screen appears as a single mound, particle-like. The first mystery of quantum mechanics arrives when we open that second slit. The results now produce something very similar to the interference pattern obtained when considering wave-like behaviors (Fig. 1.1). Rather than having two bands where the photons have gone through the two slits (Fig. 1.2), as described by the grain of sand experiment, the photons have gone through the slits behaving like waves. If we did not know anything about the photons, we could assume that there is some force between them that allows them to coordinate their actions which could give rise to the interference pattern. However, we could now adjust the experiment to try and force this understanding. Therefore, instead of sending the photons through all at once, they are sent one at a time, leaving enough of a time interval between each photon to allow those that can pass through the slits and reach the back screen. If we run the experiment slowly, gradually we can visualize the photons passing through the slits and hitting the back screen one at a time. At first, they appear to be randomly arriving at the screen, but as time goes on, that same interference wave-like pattern appears. Consequently, each photon by some means is influencing a small part to the complete wave-like behavior.

On the surface of things, this is an extremely odd function. As the photons pass through a single slit, they produce a particle-like pattern, but if they pass through two slits, even individually, they produce a wave-like pattern. Let us again adjust the



Fig. 1.1 (Top) A computational output of the interference pattern generated in Young's double slit experiment. The x-axis is dimensionless in this case to demonstrate the produced pattern. (Bottom) The computer simulation illustrating light propagating from the point source and impinging on the screen with two slits open. The two splits act like independent point source that interfere with one another. This image is intended to simplify the notion of interference phenomenon and only produces a relative distribution of light



Fig. 1.2 (Left) Two-mound example of sand granules traversing through two slits suggestive of particle-like behavior. (Right) Single-mound pattern profile produced from a photon traveling through a single slit. The x-axes are relative to the open slits

experiment to observe the photon to see the path it follows by placing a detector at one of the slits. If the photon goes through the slit with the detector there will be a record; if it does not then the assumption is that the photon must have gone through the other slit. Like the previous setup, we send the photons through one at a time; 50% of the time the detector will record the photon, meaning the other 50% of photons must have gone through the other slit. Now the only adjustment made to the experiment is the addition of a detector and as such it has detected the photons that have either gone through one slit or the other. Interestingly though, the results are now different and present in a particle-like pattern (Fig. 1.2), two distinct mounds. Finally, if we adjust the experimental again, but this time switch the detector off but leave it in place. When we run the experiment again, we obtain a wave-like pattern, almost as if the photons are aware that observation of their paths is no longer happening.

This is the complexity and puzzling properties of wave-particle duality. The act of observation can influence the result. Young's experiment demonstrates that each photon *must be aware* of both slits, and the presence of the detector, and must travel through both slits at the same time when unobserved and travel through only one or the other when being observed. Clearly, our knowledge of light is incomplete, and the wave and particle description are both just models of an incomplete picture.

For microscopy purposes, the wave-particle duality is subjugated in electron microscopy [4], *transmission and scanning electron microscopy*, and *scanning tunneling microscopy*. The electrons are used to generate small wavelengths that can be used to observe and distinguish much smaller features than what visible microscopy can achieve. However, the penetration depth of electron microscopes is unable to resolve sub-surface characteristics.

1.3 Polarization

Light waves propagate in a transverse manner, with the electric and magnetic fields oscillating perpendicular to the propagation direction of light. Additionally, the electric and magnetic fields are perpendicular to one another. When all components of the electric fields oscillate in a parallel style, the wave is said to be *linearly* polarized. The plane of polarization can be grouped by the *transverse electric* (*TE* or *s*-) and the *transverse magnetic* (*TM* or *p*-), depending on which field is absent from the direction of propagation (Fig. 1.3).

The electric field directions can also spiral along the propagation line and are known as *circular* polarization which can exist as left-handed (anticlockwise rotation) or right-handed (clockwise rotation) polarizations and maintain constant magnitude in all directions of rotation. *Elliptical* polarization can also exist but does not manage to maintain a constant magnitude due to a phase quadrature component.

Natural light (sunlight) and man-made light (light bulbs) produce unpolarized light. The unpolarized light provides a random mixture of all possible polarizations, known as *incoherent* light, which do not contain phonons with the same frequency or phase. Opposing this, *coherent* light (laser) is a beam that contains the same, or a narrow band or similar frequencies, phase, and polarization. Incoherent light can become partially polarized upon reflection.

1.4 Refraction

Snell's law formulates the relationship between the angle of incidence and refraction when light meets an interface between two mediums with different refractive indices, n_1 and n_2 . Figure 1.4 provides an example of how light rays refract at the interface, assuming $n_1 < n_2$. Since the velocity is lower in the second medium, the angle of refraction β is less than the angle of incidence α . Therefore, the relative velocity of light in the medium can be regarded as the inverse refractive index.



Fig. 1.3 Representation of linearly polarized light



Fig. 1.4 The refraction path of light entering a transparent material where $n_1 < n_2$

Snell's law permits that the path of light takes the fastest route between any two points. In comparison to the dashed line in Fig. 1.4, the solid line represents the path that the light ray has take between points A and B. As such, it can be observed that the light trails a further distance in the upper medium (n_1) , due to a lower index equaling a higher velocity, than the lower medium (n_2) that has a higher refractive index resulting in a lower velocity. Thus, AO > AC and OB < CB.

The refractive index of a given material is governed by the permittivity and permeability. These material parameters describe the density of the electric and magnetic dipole moments that have been induced by an external field. However, at optical frequencies, the magnetic component is neglected and described by unity. As such, the refractive index becomes a product of the permittivity. Thus, nonmetallic and semi-conductive materials are given the name "dielectrics." The refractive index for dielectric materials often decreases with wavelength and is called *dispersion*. However, there are spectral absorption bands where the index steeply increases with wavelength, and this is referred to as *anomalous dispersion*. Generally, the refractive index for dielectric materials is given by a real number. Yet, at the anomalous regions, they do display a complex, two-dimensional number.

In some special case materials, more notably crystals and some biological material [5] (*chitin for example*), the refractive index becomes anisotropic and varies conditionally on the direction and polarization of light. This term is known as *birefringence* and is an important consideration when studying cellular plant biology and other lengthened molecules. Isotropic materials (*with refractive index properties the same in all directions*) may convert to birefringent properties with external treatments. As light propagates and transmits through a birefringent material, a

phase difference between the different polarizations develops, resulting in the transmitted light becoming circular or elliptically polarized.

1.5 Reflection

Reflections can either be specular or diffusive. The specular type is the standard example of reflected rays from a smooth surface or interface. The diffusive type arises from a textured and uneven surface. Although diffuse reflection is an important discussion in biology, this subsection will focus on the more basic specular reflection at interfaces between a dielectric (nonmetallic) interface.

The angle of incidence and the angle of reflection are always identical. The quantity of reflected light is dependent on the polarization. The plane of incidence is defined by the plane in which both incident and reflected rays lie normal to the reflecting surface. As previously discussed, the polarization can be described by the electric field component. Light with an electric field parallel to the plane is denoted by \parallel , and with an electric field perpendicular to the place of incidence by \perp . Fresnel's equations, where α is the angle of incidence and equal to the angle of reflection, and β is the angle of transmission, provide the polarization reflection fractions (R_{\parallel} and R_{\perp}).

$$R_{\parallel} = \left(\frac{\tan\left(\alpha - \beta\right)}{\tan\left(\alpha + \beta\right)}\right)^{2}$$
$$R_{\perp} = \left(\frac{\sin\left(\alpha - \beta\right)}{\sin\left(\alpha + \beta\right)}\right)^{2}$$

The mean of the two ratios is the reflected fraction of unpolarized light. At normal incidence, $\alpha = \beta = 0$; thus, the above equations result in a division by zero. Therefore, another equation must be employed for normal incidence where there is no differentiation between the parallel and perpendicular cases.

$$R = \left(\frac{n_1 - n_2}{n_1 + n_2}\right)^2$$

Let us look at a glass microscope slide as an example for the latest equation. Microscope slides are often soda-lime glass which has a refractive index of $n_2 = 1.52$ and let us consider the glass slide in air, $n_1 = 1$. When the light hits the glass slide at normal incidence, $R = [(1 - 1.52)/(1 + 1.52)]^2 = 0.0426 = 4.26\%$. As the light passes through the glass and reaches the second interface (*glass to air*) the reflection percentage matches the 4.26% from the first interface (*air to glass*). Therefore, because the light passes through two interfaces of the same materials, the total transmitted light (1 = R + T) for soda-lime glass would be 91.6615%. In more practical terms, it can be estimated that clean glass slides, dishes, and plates have a reflection loss at normal incidence of approximately 8%. However, this value may



not hold true for plastics and glasses with filters or antireflection coatings. Additionally, if the glass substrates are not clean, then this 8% value would also not true (Fig. 1.5).

When examining the case of $\alpha < 90^{\circ}$, it can be found that $(R_{\parallel}/R_{\perp}) = [\cos(\alpha - \beta)/\cos(\alpha + \beta)]^2$ provides a ratio of >1, meaning $R_{\parallel} > R_{\perp}$. Consequently, light with an electric field perpendicular to the plane of incidence and parallel to the interface will be reflected more easily than its counterpart factor. This allows interfaces to be used as a polarizer. The reflected light can become entirely polarized with the condition of $\tan \alpha = n_2/n_1$. This special condition is called the *Brewster angle* and is the angle at which the \parallel polarized light becomes perfectly transmitted. Taking the soda-lime glass microscope example, the Brewster angle would be at $\tan^{-1}(1.52/1) = 56.7^{\circ}$.

According to Snell's law, if a light ray hits a flat interface from a high index medium to a low index medium at an increasing oblique angle, the reflection will eventually be parallel to the interface and there will be total reflection. The *critical angle* is the minimum angle of incidence at which entire reflection occurs. For example, if light was exiting a soda-lime glass microscope slide at an angle greater than critical angle, then light would be guiding and tunnel between the two interfaces. The critical angle in this case would be at $\sin^{-1}(1/1.52) = 41.1^{\circ}$.

1.6 Light Scattering

While reflection and refraction are strictly both types of scattering, the term scattering is used in a broader sense to illustrate phenomena that tend to change the order of light propagation to a random state [6]. There are three types of scattering that can be identified. The three types are *Mie scattering, Rayleigh scattering, and Raman scattering.*

Particles of the same size as the wavelength of the light while displaying a refractive index different from its surrounding generate Mie scattering. Due to the barriers between cells and the different portions of the cell make-up, almost all animal and plant tissue produce a strong Mie scattering effect.

When the scattering particle is smaller than the wavelength, Rayleigh scattering occurs. The fourth power of the wavelength of light has an inverse relationship, $1/\lambda^4$, with this type of scattering. All matter has a natural oscillating frequency that results in absorption and most substances having a strong absorption band in the far ultraviolet region. The most common natural example of Rayleigh scatter explains why the sky is blue.

Unlike Mie and Rayleigh scattering where the scattered wavelength remains unchanged, Raman scattering operates slightly different. Under Raman scattering one of two things happens, either some additional energy is taken up by the particle or the scattering particle gives off part of the photon energy. The energy disparities between vibrational states in the particle correspond to the quantity of energy taken up or given out. Raman scattering applications for a biological setting can be found in fluorescence analysis.

1.7 Diffraction and Its Limits

It is often thought that when there is nothing in its path, light travels in a straight line. However, as previously mentioned, Young's double slit experiment demonstrated that this is not always the case. Diffraction is a term that describes a variety of phenomena that occurs when a wave collides with an obstruction or an opening. It is described as the bending of waves around the corners of a barrier or through an aperture into the region of the obstacle's geometrical shadow. This facilitates the diffracting item or aperture effectively becoming the propagating wave's secondary source. Understanding some biological phenomena, such as biological crystallography [7], necessitates taking diffraction into account. Optical resolution imaging can be restricted by such issues like imperfections in the lenses or lens misalignments. Nonetheless, due to diffraction, there is an elementary limit to the resolution of any optical systems [8]. The resolution of an optical system is comparative to the size of the objective, and inversely comparative to the wavelength of the observed light.

The resolution limit to far-field objects was initially determined by Ernst Abbe in 1873. Abbe identified that light waves cannot be detained to a minimum resolvable distance in order to image anything less than one half of the input wavelength. This led to the formulation of the optical diffraction limit.

$$d_{\min} = \frac{\lambda}{2 N.A.},$$

where *d* is the minimum resolvable distance, λ is the input wavelength of light, and *N*.*A* is the numerical aperture of the optical system. The numerical aperture is usually stated on the objective of a microscope, making it an easy calculation to estimate the resolution of the optical system.

The theory surrounding the diffraction limit is founded on the Heisenberg uncertainty principle. The Heisenberg uncertainty principle engages the position and moment of a photon.

Classic physics described the free propagation waves and how they intimately relate to the diffraction limit in the far-field regions. With relation to near-field optics, there are two primary aspects that establish the resolution limit, the first being diffraction and the second being the loss of evanescent waves in the far-field regions. Evanescent waves develop at the border of two dissimilar mediums that have different refractive indices. These waves decompose significantly within a distance of a small number of wavelengths. This means that they are only detectable in the areas close to the interface of the mediums.

The evanescent waves transmit the sub-diffraction limited information, and the amplitudes of these evanescent waves weaken hastily in no less than one direction. As such, the diffraction limit could diminish if the evanescent waves become significant; this is known as super-resolution imaging [9].

For optical microscopy much of the "tiny" world is hidden. The current resolution limit for an optical microscope, unassisted from super-resolution technology, is about 200 nm. This makes live observations for ribosome, cytoskeleton, cell wall thickness, virus', proteins, lipids, atoms, and much more impossible to view with a conventional optical microscope setup.

The numerical aperture of a system can be improved with immersion oil placed between the objective and the imaging object [10]. With a high-quality immersion oil, an N.A of 1.51 can be achieved. Taking into account the point spread functions coefficient of 1.22, which describes the response of an imaging system to a point object, the resolution of an optical microscope at 550 nm would be $d = 1.22 \times (550 \text{ nm}/(2 \times 1.51)) = 222 \text{ nm}$. In most biological specimens, using a longer wavelength like infrared has the advantage of better penetration and less scattering but lower resolution [11].

1.8 Photon Absorption, Fluorescence, and Stimulated Emission

Photon absorption is the process by which matter absorbs a photon's energy and converts it into the absorber's internal energy which is often dissipated through heat or a release of a photon. Absorption is achieved when bound electrons in an atom become excited as the photon's frequency matches the natural oscillation, or resonant, frequency of a particular material. This results in the light intensity attenuating as the wave propagates through the medium. Wave absorption is normally independent of the intensity under linear absorbing behaviors (Fig. 1.6). However, under some circumstances, particularly in the realm of optics, a material's transparency can fluctuate as a function of wave intensity resulting in saturable absorption. This process is a nonlinear effect.

The simultaneous absorption of two photons of the same or difference frequencies to excite a molecule or atom from one state, typically the ground state, to a higher energy is known as *two-photon absorption* (TPA) [12]. This is a nonlinear absorption effect. The change in energy from the lower and upper state is equal to or less than the sum of the photon energies of the two absorbed photons. This phenomenon is achieved by creating a virtual state between the ground and excited states where excitation is achieved by two separate one-photon transitions (Fig. 1.7). An example of applications involving TPA can be found in



Fig. 1.6 A schematic diagram showing an overview of electromagnetic radiation absorption. As a white light source illuminates the sample, the photons that match the correct energy gap are absorbed and excited to a higher energy state. The other photons remain unaltered and transmit through the material



Fig. 1.7 (Left) The energy transition diagram for stimulated emission. Starting from a high energy state, as the photon with the correct energy interacts with the material, it stimulated the electron to decay and emit an identical photon. (Right) The energy levels for a two-photon absorbing system demonstrating the virtual state

fluorescent molecules where the excited state electrons decay to a lower energy state via spontaneous photon emission.

Spontaneous emission is the process where an excited energy state transitions to a lower energy state and releases the energy in the form of a photon. Emission can also be a stimulated process where a delivered photon interacts with a pre-excited electron to force or stimulate the electron to drop to a lower energy level. The stimulated process obtains its original photon and creates a new photon identical to the input photon, in wavelength, polarization, and direction of travel. The emission of photons from stimulated emission is a consequence of the conservation of energy and the wavelengths are well defined by quantum theory. As an electron transitions from a higher energy level to a lower energy level, the difference in energy between the two transition energy levels is released to conserve the energy of the system. The energy difference between the two levels regulates the emitted wavelength. This is the fundamental process of how lasers operate.

In terms of fluorescence, the electron absorbs a high-energy photon that is excited from the ground state. The electron then relaxes vibrationally and drops state levels through non-radiative transitions. The electron is still in a high state than the pre-absorption state, but a lower energy state compared to post-absorption. As such, when the electron falls back to the ground state, the material fluoresces at a longer wavelength (Fig. 1.8). Fluorescence has become an important function for the field of microscopy such as the use in fluorescence-lifetime imaging microscopy [13]

1.9 Filtering Light

It is often the case with optical instruments that in many circumstances the user may not want to use light directly from the light source. It may be beneficial to delete particular sections of the spectrum or pick only a restricted spectral band, or select





light with a specific polarization, or modulate the light in time, such as swiftly changing from darkness to light or obtaining a sequence of light pulses. All this can be achieved through optical filters, which provide use for a number of applications including microscopy, spectroscopy, chemical analysis, machine vision, sensor protection, and more. A variety of filter types are available and used according to the application requirements.

Similar to other optical components, filters possess many of the same specifications. However, there are several specifications unique to filters and require an understanding in order to determine what filter is suitable for a particular application. These include *central wavelength*, *bandwidth*, *full width-half maximum*, *blocking range*, *slope*, *optical density*, and *cut-off and cut-on wavelengths*. There are two main filter types to consider, *absorption* and *interference filters*.

The light-absorbing properties of some substances can be utilized to exploit blocking unwanted light. Light that is prevented through absorption is retained within the filter rather than reflecting off it. For example, certain types of glass can be excellent absorbers of UV light. Another common absorbent is cellulose acetate, although can be unstable and does bleach with time. Water can be another useful absorbing filter, particularly for infrared radiation. Potassium dichromate solutions are superb in absorbing light with wavelengths shorter than 500 nm. Although this is a useful solution, particularly for studying fluorescence, the liquid is also carcinogenic, and caution should be upheld.

Interference filters, often referred to dichroic filters, work by reflecting undesired wavelengths while transmitting the desired portion of the spectrum. The interference characteristic of light waves is exploited by adding numerous layers of material with variable indices of refraction. Only light of a specified angle and wavelength will constructively interfere with the incoming beam and pass through the material in interference filters, while all other light will destructively interfere and reflect off the material. These types of filters have much great flexibility in design, but the theory



Fig. 1.9 Basic diagram outlining some of the fundamentals discussed in this chapter

can be complicated. Thankfully, there are many experts and companies that offer a range of dichroic filters.

Some solutions provide far superior filtering but may come at a cost. For desired optical qualities, thick layers may be required, and liquid filters may become large. Additionally, some of the most valuable-colored compounds are cancerous or hazardous in other ways. Therefore, great precaution is needed when selecting a desirable filter, particularly with absorbing ones. The more costly and expensive interference filters are often chosen due to these factors. However, the dichroic filters are extremely angle sensitive, unlike absorbing filters.

Take-Home Message

- Light is a beam of energy that travels at the universal speed limit.
- Light is not only the visible light we see but extends the entire electromagnetic spectrum from radio waves down to gamma rays.
- Light is a wave of altenating electric and magnetic fields.
- Light can be described as both a wave and a particle.
- Different wavelengths of light interact differently with matter (Fig. 1.9). For example, how plants turn sunlight into energy through the process of photosynthesis.
- Light can be manipulated for our advantage through reflection, refraction, polarization control, and scattering.
- Optical systems such as microscopes have an optical resolution limit that restricts the resolvable features. For example, molecules are undetectable by white light micrscopy.
- We can take advantage of optical filters and fluorescence to advance our imaging quality and contrast.

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