MPHY3886, MPHYM886, MPHYG886
OPTICS IN MEDICINE
Introduction to Laser-Tissue Interactions

Ben Cox

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Contents

1 Introduction

Interactions between light and biological tissue occur all the time. Sunburn and tanning are commonly experienced, but do we known what causes them? Is sunburn really a burn? Why do I tan when I warm myself in the sun, but not when I warm myself by the fire? How does clear sunblock work?[1]

Light-tissue interactions are essential for life. Plants, algae and bacteria require sunlight to photosynthesise sugars such as glucose. Humans too depend on photochemical reactions, eg. exposure to ultraviolet light is vital for the production of vitamin D in the skin. However, light can also have undesirable effects such as causing cancers. eg. UV damage to the DNA in melanocytes can lead to melanoma, a type of skin cancer.

In the examples above the sun is the source of light. In Advantium® ovens sold by the company GE - which use light to cook food - the source of light is two halogen bulbs. When using light for medical procedures it needs to be very well controlled, and so laser light sources are used. These lectures explore some of the ways in which laser light and biological tissue interact, and how and why lasers are used in various medical procedures. We want to be able to answer questions like:

When a laser beam is used as a scalpel in surgery, how do you know how deep it will go? Why doesn't it go all the way through?

How does laser eye surgery work and is it safe?

When a laser is used to remove a tattoo does it leave a scar?

It is well known that sunlight can cause cancers, but how can light be used to *treat* cancers?

How can lasers be used to image the network of subsurface capillaries?

Is it possible to cook something using a laser pointer?

1.1 Medical Treatments using Lasers

Although we will categorise tissue-laser interactions into just five mechanisms, the list of medical applications is long and growing. Here is a brief and non-exhaustive list of some of those uses. An indication of the main physical mechanism at work is given, although this is usually an oversimplification as there will often be more than one mechanism at work, and for some of the procedures the mechanism is not yet fully understood. Some of these applications are described in greater detail in further chapters.

[1] (1)Sunburn is not a burn - the skin is not being oxidised - but is an inflammatory reaction which forms part of the immune response to the killing of skin cells by ultraviolet (UV) radiation. (2) Light from a fire contains predominantly infrared radiation whereas sunlight contains UV light as well, which has more energetic photons. It is thus able to stimulate melanocytes, cells that produce the brown pigment melanin, into producing more of it. (3) Sunblocks typically contain particles that reflect UV radiation, such as titanium dioxide. When the particles are smaller than 100 nm or so they are transparent at visible wavelengths, but still block UV light.

Ophthalmology

- Cornea: myopia, hyperopia and astigmatism correction. (Photoablation)
- Retina: detachment, retinopathy, central vein occlusion, senile macula degeneration, retinal tumours. (Photothermal, coagulation)
- Lens capsulotomy to treat secondary cataracts. (Plasma-induced ablation)

Dermatology

- Removal of skin tumours, external ulcers and warts. (Photothermal, vaporization, photodynamic therapy)
- Treatment of port wine stain (*naevi flammei*) and haemangioma. (Photothermal, selective photothermolysis)
- Hair removal. (Selective photothermolysis)
- Tattoo removal. (Selective photothermolysis)

Oncology

- Tumour ablation. (Photothermal, vaporization)
- Photodynamic therapy. (Photochemical)

Neurosurgery

- cutting, vapourising, coagulation without mechanical contact. (Photothermal, photoablation)

Angioplasty

- Atherosclerosis: removal of blood vessel plaque. (Photothermal)

Orthopaedics

- Cartilage and bone ablation. (Photothermal)

Gastroenterology

- Endoscopic surgery to treat gastrointestinal haemorrhage; beam guided through water jet. (Photothermal)
- Treatment for benign or malignant stenoses, a narrowing of a tube eg. oesophagus. (Photothermal)

Ear, Nose and Throat (ENT, Otorhinolaryngology)

- Microsurgery of the larynx. (Photothermal)
- Stapedotomy: making a hole in the stapes to improve hearing. (Photothermal)

2 Light-Tissue Interactions

2.1 De-excitation Pathways

Once a molecule has absorbed a photon, one of several things could happen:

Radiative and non-radiative relaxation. Imagine an excited molecule that is alone, without any other nearby molecules to interact with. In this case, two things could happen. First, the energy gained by absorbing the photon, and initially stored in one mode, will begin to be shared out between all the modes in a *non-radiative* process of *intramolecular redistribution* until the molecule is in equilibrium (according to the equipartition theorem). However, the molecule could also jump abruptly to a lower energy state by emitting a photon. If the *radiative lifetime* of the molecule is shorter than the redistribution time, then it is likely that a photon will be emitted before the process of intramolecular redistribution has completed. As some redistribution will always take place before a photon is emitted, the energy of the reradiated photon will always be lower than the absorbed photon. There are two possible *radiative processes: fluorescence and phosphorescence.* During fluorescence there is a transition from a state to a similar state, eg. singlet-singlet, and is typically fast (ns or shorter). Phosphorescence occurs after an intramolecular *inter-system crossing* has taken place, so the transition accompanying the radiation typically involves a change from a triplet to a singlet state which is much less likely to occur (according to quantum mechanics), and so the radiation is of lower energy and occurs over a much longer timescale (ms, seconds or even longer). All mechanisms that are not *radiative* are by default *non-radiative.*

When there are other molecules nearby, then there are more things that can happen, sometimes called, somewhat melodramatically, the different 'fates of excited species':

Photochemical reactions. When the light absorption gives rise to an electronic transition, the more energetic electron will, on average, orbit the nuclei at a greater distance. As the attractive nuclear force falls off rapidly with distance, the electron will be less tightly bound, and will be able to form a chemical bond with another molecule more readily. This is the basis of *photochemistry.*

Thermalisation, collisional relaxation. While an excited molecule is undergoing intramolecular redistribution it might collide with another molecule. Some of the vibrational energy in the excited molecule will transferred to the colliding molecule as *translational kinetic energy.* Molecular translational kinetic energy is what appears at a macroscopic level as a temperature rise so leads to *photothermal effects.* This process of *collisional relaxation* will thereby *thermalise* the absorbed photon energy in a matter of picoseconds, although the resulting macroscopic thermal effects occur over very much longer timescales (ms to s).

2.2 Plasma Formation

One mechanism not yet mentioned that has important clinical applications is plasma generation. When the laser beam is sufficiently intense - sufficiently tightly focussed - its electric

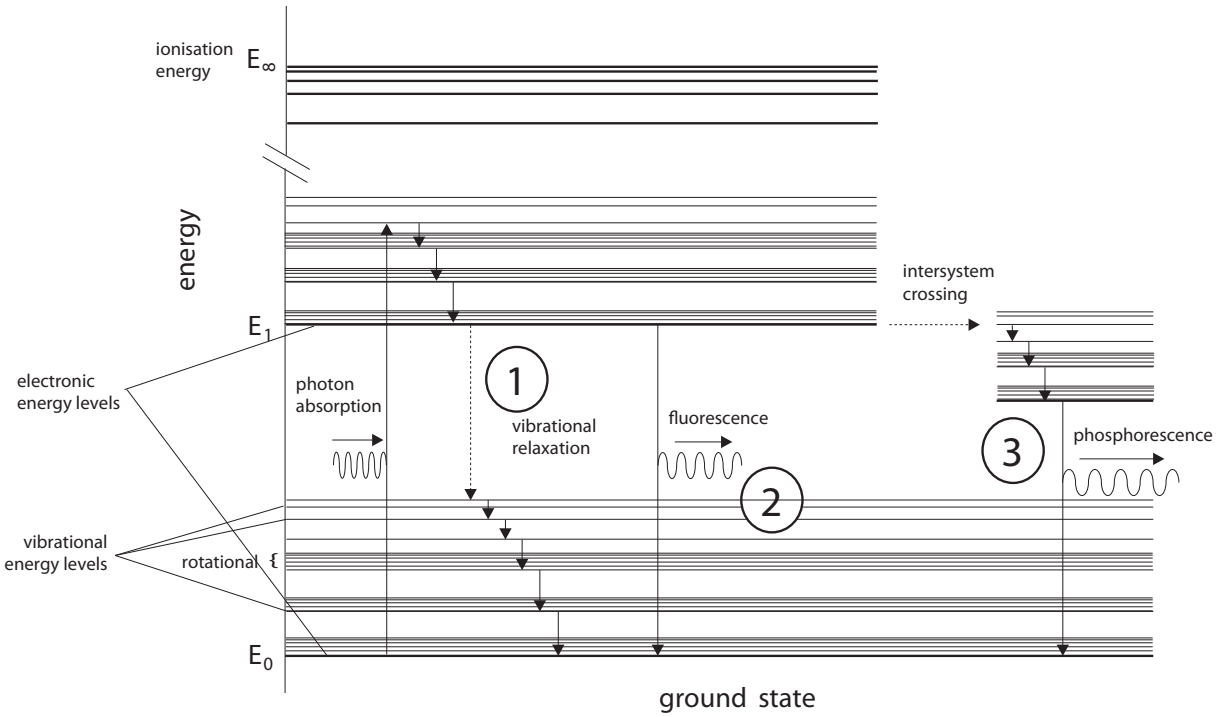


Figure 1: Three of the ways in which the excited state can return to the ground state: thermalisation (via collisional and vibrational relaxation), fluorescence (short-lived photon emission) and phosphorescence (long-lived photon emission).

field can accelerate a free electron (ie. one not bound to a molecule) to such a speed, ie. kinetic energy, that it can ionise a molecule with which it collides without itself becoming bound. This results in two free electrons. These are now both accelerated by the intense electric field, and collide with and ionise more molecules, resulting in four free electrons, and so on. This chain reaction leads to a plasma, which is the name given to a soup of free electrons and ionised molecules. In order for the chain reaction to 'go', the electric field must be so intense that the rate at which electrons are 'freed', ie. ionised, is greater than the rate at which they become re-bound to ions. Because the translational kinetic energy of free electrons is not quantised in the way that bound electrons have quantised energy levels, the plasma can absorb all incoming radiation whatever the wavelength. This can be useful in applications for 'mopping up' spare photons. It is sometimes known as *plasma shielding*.

Where did the first free electron, ie. the one that started off the chain reaction, come from? It may just have been a free electron in the right place at the right time (sometimes called 'lucky' electrons). It is more likely that it was emitted just prior to being accelerated, either through ionisation following two- or multi-photon absorption, or by thermionic emission. (This is analogous to evaporation in a liquid.)

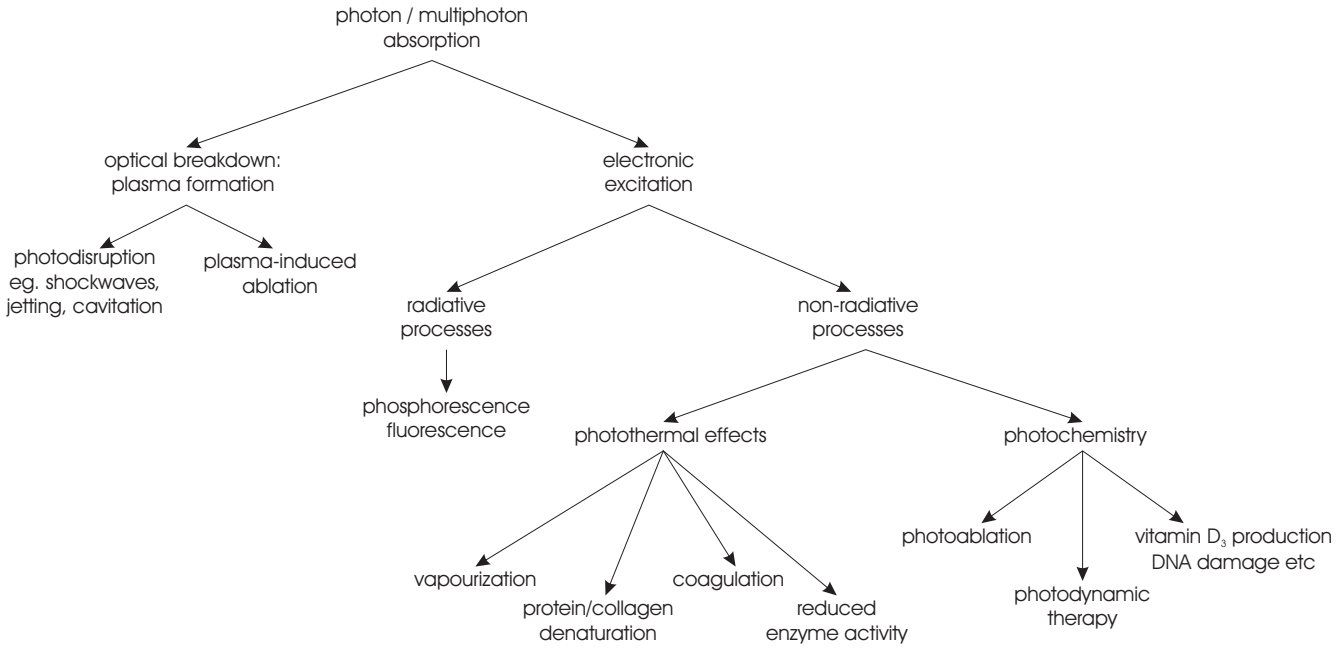


Figure 2: Some of the mechanisms and effects of light-tissue interactions.

2.3 Types of Interactions

There are many different mechanisms by which laser light can interact with tissue, and these have been categorised in a number of different ways by different authors. For the purposes of these lectures, the most common interaction mechanisms for therapeutic and surgical applications will be divided into five broad classes:

1. *Photochemical reactions*: when a molecule absorbs a photon of sufficient energy, the energy can be transferred to one of the molecule's electrons. An electron with higher energy can more easily escape the nuclear forces keeping it close to the nucleus, and so excited molecules (which are molecules with an electron in a higher energy state) are more likely to undergo chemical reactions (exchanging or sharing of electrons) with other molecules. In photodynamic therapy, for instance, a photosensitising drug (a concoction of molecules which, when they absorb light, cause reactive oxygen species to form) is used to cause necrosis (cell death) and apoptosis ('programmed' cell death). Photodynamic therapy is increasingly widely used in oncology to destroy cancerous tumours.

2. In *photothermal* interactions, the energy of the photons absorbed by chromophores (a term used to refer to any light-absorbing molecules) is converted into heat energy via molecular vibrations and collisions, which can cause a range of thermal effects from tissue coagulation to vaporization. Applications include tissue cutting and welding in laser surgery, and photoacoustic imaging.

3. In *photoablation*, high-energy, ultraviolet (UV) photons are absorbed by electrons, raising them from a lower energy 'bonding' orbital to a higher energy 'non-bonding' orbital, thereby causing virtually immediate dissociation of the molecules. This naturally leads to a rapid expansion of the irradiated volume and ejection of the tissue from the surface. This is used in eye (corneal) surgery, among other applications.

4. In *plasma-induced photoablation* a free (sometimes called 'lucky') electron is accelerated by the intense electric field which is found in the vicinity of a tightly focussed laser beam. When this very energetic electron collides with a molecule, it gives up some of its energy to the molecule. When sufficient energy is transferred to free a bound electron, a chain reaction of similar collisions is initiated, resulting in a plasma: a soup of ions and free electrons. One application of this is in lens capsulotomy to treat secondary cataracts.

5. The final set of related mechanisms, grouped under the term *photodisruption*, are the mechanical effects that can accompany plasma generation, such as bubble formation, cavitation, jetting and shockwaves. These can be used in lithotripsy (breaking up kidney or gall stones), for example.

2.4 Selecting an Interaction Mechanism

What determines which of these five interaction mechanisms will dominate in a particular case? The dominant mechanism will depend on

1. the type of molecules the tissue is made of and contains. (These determine the energy levels - the energies of photons that can be absorbed - and the available de-excitation pathways, ie. the routes through which the energy leaves the state into which it was absorbed, to end up as heat or perhaps another photon.)

2. the frequency (or wavelength) of the light, ie. the energy associated with each individual photon,

3. the power per unit area delivered by the laser,

4. the duration of the illumination, and repetition rate of the pulses for a pulsed laser.

Because different interaction mechanisms dominate under different conditions (photoablation requires UV light, photodisruption requires very short duration pulses, etc), by carefully choosing the laser characteristics the interaction can be restricted to a specific mechanism, and therefore a specific effect on the tissue. Lasers are therefore useful for medical applications because:

a. the energy of the photons can be chosen, as each type of laser will emit photons of only one energy (one frequency or wavelength),

b. the power can be carefully controlled over a wide range of fluence rates,

c. the beam shape can be well controlled (focussed, collimated, etc), and

d. the duration of the laser pulses can range from as-long-as-you-like to less than 100 femtoseconds. (100 femtoseconds is really quite a short time. It is about the time it takes light to travel the thickness of a human hair.)

Fig. 3 shows how the five interaction mechanisms depend on the duration of the light exposure and the irradiance (fluence rate), ie. the light energy delivered per unit area per unit time, the power per unit area, in W/cm^2. This is only a rough guide, of course, but it helps us put the mechanisms into some sort of order. Notice that both axes are log-axes, ie. the log of the irradiance increases linearly on the vertical axis, and the log of the time increases linearly on the horizontal axis. To get a feel for the magnitude of these irradiances, the average irradiance at the surface of the earth due to the sun is 0.14 W/cm^2, and the irradiance from a standard 1mW laser pointer with a spot of radius 1 mm is $0.001/(\pi 0.001^2) = 318$ W/m^2 = 0.03 W/cm^2. For laser eye surgery the irradiance can be as much as *thirteen orders of magnitude higher* at 10^{12} W/cm^2.

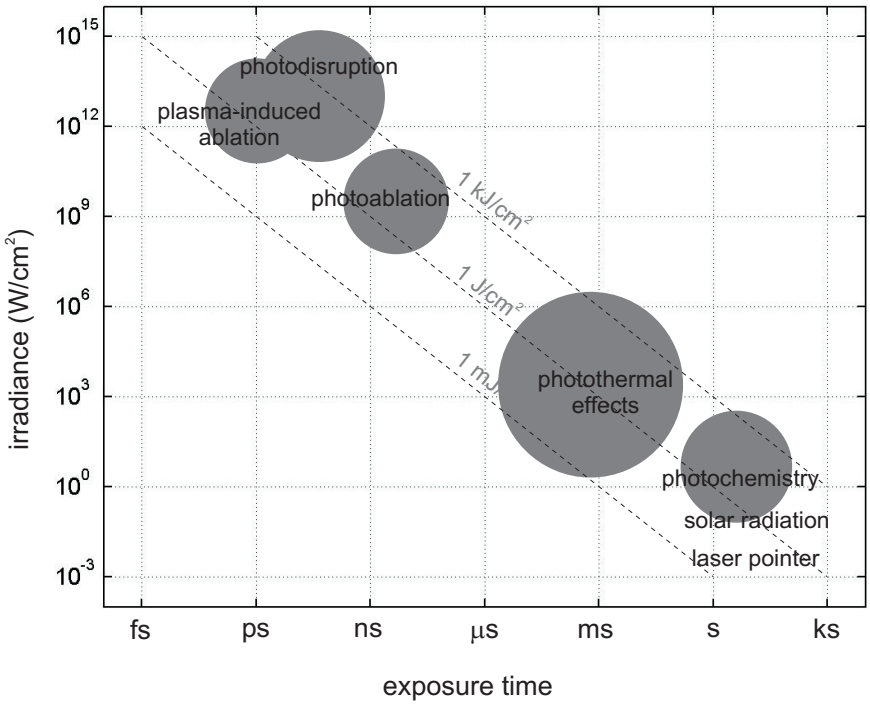


Figure 3: An overview of the different types of laser-tissue interaction, and the irradiance and exposure durations at which they dominate.

3 Photodynamic Therapy

3.1 Photochemistry

A photochemical reaction is one in which light initiates a chemical reaction. Perhaps the best known photochemical reaction is photosynthesis, the set of reactions by which plants and some bacteria turn water and carbon dioxide into carbohydrates - such as glucose - and oxygen. Without photochemical reactions, most of the life on Earth would not exist.

For humans, the production of vitamin D_3 from cholesterol in the skin is an important photochemical reaction. Vitamin D's most important role is to promote bone formation; it also has other uses, such as enhancing calcium transport. A lack of sunlight can lead to problems with bone mineralisation causing ricketts and osteomalacia.

The main use of photochemistry in medicine is in photodynamic therapy (PDT), which is widely used to treat cancers. In photodynamic therapy, a photosensitiser - a light-activated drug - is injected, drunk, or otherwise introduced into the body, and left to accumulate in tumour tissue. This tissue is then illuminated with a specific wavelength of light, which leads to necrosis and apoptosis.

3.2 History

- Ancient Egyptians (apparently) used light to treat skin complaints, such as vitiligo and psoriasis.
- 1900. Oscar Raab noticed that paramecia (unicellular organisms), that were unaffected by light or acridine orange separately, were killed when the two were combined.
- 1903. Niels Finsen won Nobel prize for treating smallpox and cutaneous tuberculosis with 'phototherapy'.
- 1907. Herman von Tappeiner uses the term 'photodynamic' for the treatment of skin tumours using eosin and white light.
- 1913. Meyer-Betz used porphyrins to demonstrate PDT on his own hands and experienced a systemic inflammatory reaction.
- 1955. Haematoporphyrin derivative (HPD), developed by Schwartz, more toxic than haematoporphyrin.
- 1960. Lipson and Baldes notice that HPD accumulates in tumours and so can be used to detect them because it fluoresces.
- 1976. PDT used in bladder cancer.
- 1993. First PDT drug (ALA) approved for use.

3.3 Clinical Procedure

In photodynamic therapy two ingredients that are non-toxic on their own - a photosensitiser and light - combine, in the *presence of oxygen* to kill cells. The procedure is shown in Fig. 4:

1. Photosensitiser injected, drunk, or topically applied to the skin.
2. Photosensitiser accumulates in tumours and mostly clears from healthy tissue.
3. Laser irradiation incident on the photosensitisers makes them generate toxic species.
4. The toxicity causes cell necrosis and apoptosis predominantly in the tumours.
5. The immune system clears up the dead cells and attacks remaining malignant cells.

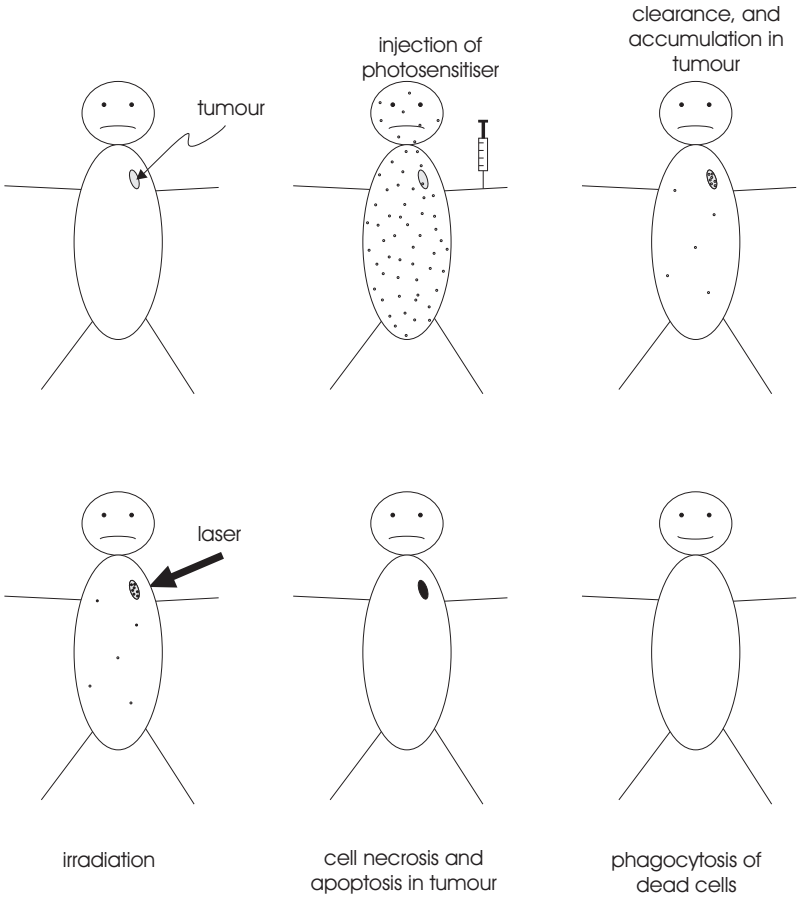


Figure 4: Photodynamic therapy procedure for cancer treatment.

3.4 Applications

There are many potential applications for PDT, but few currently used clinically. Some of the conditions for which PDT is more likely to be a standard treatment are listed below.

- very early lung cancers - a bronchoscope is used to deliver the light.
- skin - basal cell carcinoma, squamous cell carcinoma, pre-cancerous actinic keratosis (but not melanoma which metastatises too quickly and is often too pigmented to allow light to penetrate).
- oesophagus - pre-malignant (dysplastic) Barrett's oesophagus. (Currently the standard treatment is with RF ablation - electrically-induced thermal necrosis).
- head and neck tumours - good cosmetic results, reduced scarring compared to conventional surgery.
- arterial disease - prevents restenosis (re-narrowing of an artery) after angioplasty.
- 'mopping up' following radiotherapy.
- Ophthalmology - age-related macular degeneration (AMD)

3.5 Mechanisms and Pathways

PDT works by generating highly toxic *reactive oxygen species* (ROS) to kill cells. The term 'reactive oxygen species' encompasses a number of different molecules, including peroxides, free radicals, oxygen ions, etc. all of which are highly reacting, and therefore react with, and damage, cellular components and the cell membrane. This causes both necrosis and apoptosis (the two known types of cell death).

When a photosensitive molecule is in its singlet ground state, call it ${}^1P$, and it absorbs a photon, it is put into an excited singlet state, ${}^1P^*$. Then, one of several things can happen:

1. Non-radiative singlet decay, ${}^1P^* \rightarrow {}^1P$, with the energy being lost through vibrational relaxation. (Not what we are interested in here.)
2. Radiative singlet decay, ${}^1P^* \rightarrow {}^1P + h\nu$, i.e. fluorescence, lower energy photon emitted a few nanoseconds after absorption. Photosensitisers often exhibit fluorescence and so can be used to *detect* as well as treat tumours.
3. Conversion to a triplet state via an intersystem crossing.

If one of the energy levels for P in an excited singlet state, ${}^1P^*$, happens to be similar to an energy level with P in an excited triplet state, ${}^3P^*$, then the molecule may convert to this triplet state: this is called an intersystem crossing. The triplet state is a relatively stable and long-lived state, and can undergo reactions that result in ROS.

There are thought to be two main types of reaction, imaginatively named Type I and Type II. These are shown in Fig. 5. The Type II reaction, which creates excited singlet oxygen

$^1O_2^*$, a very reactive species, from the usual triplet form $^3O_2$ is considered the most important for photodynamic therapy. The spins in this reaction are flipped as follows

$$^3P^*(\uparrow\uparrow) + {}^3O_2(\uparrow\uparrow) \rightarrow {}^1P(\uparrow\downarrow) + {}^1O_2^*(\uparrow\downarrow) \tag{1}$$

The generation of cytotoxic ROS in PDT *requires the presence of oxygen*, eg. if the tissue is clamped to prevent blood from reoxygenating the tissue so the tissue becomes hypoxic (lack of oxygen) then photodynamic therapy does not work.

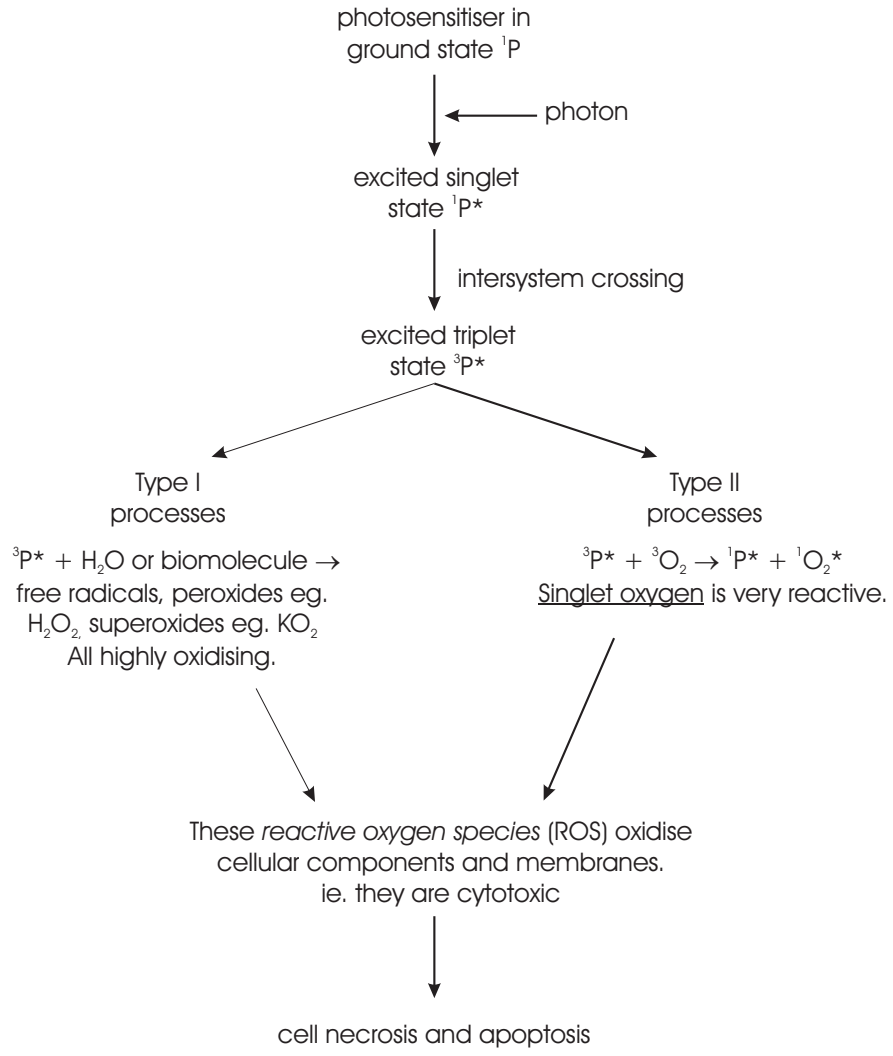


Figure 5: Mechanisms of photodynamic therapy.

3.6 Photosensitisers

There are several classes of photosensitiser, with porphyrin-related photosensitisers being the most important and the most widely used. The details of three key photosensitisers are given below. New candidate photosensitisers are becoming available (eg. Visudyne, WST11, Lu-Tex) and research continues on novel and improved photosensitisers for particular applications, often treating tumours.

Ideal photosensitiser The holy grail is a photosensitiser that is:

- chemically pure and of known composition,
- only cytotoxic in the presence of light,
- highly efficient, ie. high quantum yield (many ROS generated per photon),
- activated in the range 630-800 nm where tissue is most transparent (so can reach deep tissue) and photons are still energetic enough to generate ROS,
- rapidly cleared from the body (so quality of life is not unduly affected and repeat treatments are possible),
- preferentially retained by the target tissue, eg. tumour (not always important in practice, but clearly a useful property).

Hematoporphyrin derivative (porfimer sodium, HpD, $\lambda_{\mathbf{act}} = 630$ nm,$\epsilon_M \approx 3000$ $\mathbf{cm^{-1}M^{-1}}$) was the first photosensitiser used in PDT. To obtain hematoporphyrin derivative, hematoporphyrin from calf blood is treated with acetic and sulphuric acids, filtered and then neutralised with sodium acetate. HpD contains several different porphyrins. Photofrin® is a partially purifed form of HpD from which some of the less active porphyrins have been removed, and was the first commercially available photosensitiser for PDT. It can take several weeks to completely clear from the body, and patients are advised to avoid direct sunlight or bright indoor lighting for 30+ days after injection. Photofrin is used to treat endobronchial and oesophagael cancers.

5-aminolaevulinic acid (ALA, $\lambda_{\mathbf{act}} = 635$ nm, $\epsilon_M \approx 5000$ $\mathbf{cm^{-1}M^{-1}}$) generates a photosensitiser endogenously (within the body) called protoporphyrin IX (PpIX). PpIX tends to accumulate in mucosal tissue because, being metabolically more active, they convert more PpIX from ALA. Other advantages of ALA are that it clears in 24-36 hours, thus reducing the duration for which the skin is light sensitive, and the sensitiser level can (at least in principle) be assessed through its fluorescence. Under the tradename Levulan® it has been used to treat basal cell carcinoma (a skin cancer), head and neck tumours, gynaecological tumours, acne and other types of cosmetic surgery. The company Photocure sells related products Hexvix®, a hexaminolevulinate solution for injection and used to treat bladder cancers, and the cream Metvix®, which is methyl aminolevulinate (MAL) used applied topically to pre-cancerous skin lesions.

Meta-tetrahydroxyphenyl chlorin (m-THPC, Foscan®, $\lambda_{\mathbf{act}} = 652$ nm, $\epsilon_M \approx 30000$ $\mathbf{cm^{-1}M^{-1}}$) is considerably more effective than Photofrin allowing much lower doses to be

used. It is also activated at a longer wavelength allowing deeper tumours to be treated. As Foscan it has been used to treat head and neck tumours, but because of its much higher efficiency much greater care must be taken to avoid sunlight and other unwanted sources of light. Another disadvantage is that its clearance rate is much slower than ALA, with patients advised to avoid bright lights for several weeks and sunbathing for perhaps 3 months.

3.7 Dosimetry

The lack of sufficiently accurate and reliable dosimetry is currently preventing PDT from being more widely used in the clinic. The aim in PDT is to kill all the cancerous cells while harming as few leaving healthy cells and as little extracellular matrix as possible. So if a PDT 'dose' of D_n is required to necrose the tissue, then the *ideal* dose is the combination of light, photosensitiser and oxygen for which

$D(\mathbf{x}) > D_n$, where $\mathbf{x}$ is a point in a region of cancerous tissue, but
$D(\mathbf{x}) < D_n$ where $\mathbf{x}$ lies in healthy tissue.

In practice, damage to some of the healthy tissue surrounding a cancerous region is acceptable - even encouraged as it will help ensure all the cancerous cells have been killed. The extracellular matrix (ECM) that remains will be repopulated with healthy cells, with less damage to the ECM than during conventional surgery, and, although some scarring can occur, usually much better cosmetic results.

1. How should the dose D be defined? How do the fluence rate, photosensitiser concentration, tissue oxygenation, etc. relate to D?
2. How does tissue damage vary with D? What values does the necrosing-dose D_n take for different types of tissue?
3. How can we know what the dose is in a particular case?
4. How can the fluence rate, etc. be adjusted to ensure the dose is ideal?

3.7.1 Explicit dosimetry: a definition of dose

In PDT, cell death is caused predominantly through reactive oxygen species (ROS) such as singlet oxygen reacting with, and thereby denaturing or breaking, the cell membrane or other cellular components crucial to the continuing function of the cell. The concentration of molecules of ROS would therefore seem to be a good place to start when looking for a measure of PDT dose. In PDT, ROS are generated through the interaction of light, oxygen and photosensitiser.

Consider a point within a piece of tissue where the photosensitiser concentration is C_{ph} moles per litre (or molar, M). If the photosensitiser has a molar *extinction* coefficient of ϵ_M then its molar *absorption* coefficient is $\mu_M = \epsilon_M \ln(10)$ $\text{cm}^{-1}\text{M}^{-1}$. If we assume that the photosensitiser is the dominant chromophore, then the absorption coefficient is

$$\mu_a = \mu_M C_{ph} \quad \text{cm}^{-1} \tag{2}$$

If the fluence rate is Φ Wcm^{-2} then the power absorbed by the photosensitiser molecules is

$$H = \Phi\mu_a \quad \text{Wcm}^{-3} \tag{3}$$

(There is a potential complication here in that some photosensitisers will bleach over time, ie. they will become less absorbing the longer they are irradiated for.) The quantum yield, also usually notated Φ so here notated Φ_{qy}, is the number of activated photosensitiser molecules per absorbed photon, then the absorption of power density H will result in

$$N_{act} = \Phi_{qy}H/h\nu \quad \text{activated molecules cm}^{-3}\text{s}^{-1} \tag{4}$$

where $h\nu$ is the energy in a photon, ν the frequency of the light and h Planck's constant. If, on average, each activated photosensitising molecule generates K ROS molecules, then the density of ROS molecules generated per second, N_{ROS}, is

$$N_{ROS} = KN_{act} \quad \text{cm}^{-3}\text{s}^{-1} \tag{5}$$

where K is called the photodynamic efficiency and will depend on the availability of oxygen molecules, ie. the tissue oxygenation. Not all of the ROS molecules will oxidise a biomolecule which is important to the survival of the cell containing it. If a proportion f of the generated ROS cause damage to a significant cellular component then the *effective oxidative damage rate* can be written as fN_{ROS}. If we take the effective oxidative damage to be a measure of dose, then putting all these equations together gives

$$D_v = fN_{ROS}t = \frac{fK\Phi_{qy}\Phi\mu_M C_{ph}t}{h\nu} \quad \text{instances of damage per unit volume} \tag{6}$$

for an exposure duration of t seconds. In radiotherapy, tissue damage is correlated with the absorbed energy per unit mass rather than per unit volume. If this is the case then the dose can be written

$$D_\rho = \frac{D_v}{\rho} \quad \text{instances of damage per unit mass} \tag{7}$$

where ρ is the mass density of the tissue in kgm^{-3}. It is currently unclear as to whether Eq. (6) or (7) represents a better measure of dose for PDT *in vivo*.

Whereas some of these quantities, eg. Φ, μ_M, t, ν, can be measured or are known, the others must be inferred from secondary measurements (such as a measurement of oxygenation to infer K, or a measurement of fluorescence to infer C_{ph}) or estimated through *ex vivo* measurements on tissue samples.

3.7.2 Implicit dosimetry

As opposed to explicit dosimetry, in which all the various quantities in Eqs. (6) or (7) are either measured or estimated through prior measurements or models, implicit dosimetry measures a phenomenon that is correlated with the degree of tissue damage, but is different from it. As most of the cell damage is assumed to be through the action of singlet oxygen molecules, a measure of these may act as an indicator of the dose. For example, the rate at which the photosensitiser Photofrin bleaches over time seems to be dependent on singlet

oxygen, so may act as a quantitative measure of the singlet oxygen concentration. In fact, for superficial treatments, the concentration of singlet oxygen can be measured through the weak phosphorescent emission at 1270 nm when singlet oxygen returns to its (triplet) ground state.

3.7.3 Adjusting the dose

Increasing the exposure duration t increases the dose everywhere uniformly. It is more important, however, to be able to vary the dose spatially to target the region of interest and avoid necrosing too much healthy tissue. A number of quantities will affect the spatial distribution of the dose.

- K, the photodynamic efficiency (or effectiveness) will depend on the type of photosensitiser as well as the concentration of oxygen within the tissue. In practice, the choice of photosensitiser will depend on a number of other factors as well as its effectiveness in generating ROS, for instance, how long it takes to clear from healthy tissue. To vary K spatially, the sensitiser may be applied to the tissue non-uniformly, ie. only to the region of interest, or the tissue oxygenation could be varied.

- C_{ph}, the concentration of the photosensitiser within the tissue will be a function both of time and of the propensity of the photosensitiser to accumulate in certain types of tissue rather than others. The time the photosensitiser takes to clear from healthy tissue and accumulate in tumours can be anything from a few hours to several days, depending on the particular drug. One way to change the dose is therefore to adjust the length of time between administration of the photosensitiser and illumination of the tissue.

- Φ, the fluence rate can be altered by changing the way the tissue is illuminated, perhaps by changing the place at which the light is introduced to the tissue. To calculate the dose accurately, it is clearly very important to be able to model how the light travels within the tissue. Computational models of light transport may be used for this purpose.

- ν, the frequency, or equivalently the optical wavelength, λ, can be varied to alter the dose because many of the terms in Eq. (6) will be wavelength dependent. However, it is usual to choose a wavelength at which tissue is most transparent, ie. where water absorption is lowest, in the red and near infrared regions of the spectrum, to permit maximum penetration of the light into the tissue.

3.7.4 Practical dosimetry

Currently, neither explicit nor implicit dosimetry are used regularly in patient care, because of the difficulties involved in measuring several of the required quantities: singlet oxygen concentration, fluence, photosensitiser concentration... and so on. Many reserch groups and clinical scientists are looking at ways to improve these measurements, and make case-by-case dosimetry for PDT practical. However, at the moment, dosimetry is based largely on previous experience, and on the results of clinical trials of defined and ethics-committee-approved protocols. For instance, part of a typical protocol for treating Barrett's oesophagus might be as follows:

Hour 1 - drink first dose of ALA, 20 mg/kg body weight
Hour 2 - drink second dose of ALA, 20 mg/kg body weight
Hour 3 - drink third dose of ALA, 20 mg/kg body weight
... Hour 7 - illuminate with green light, λ=514 nm, power density 120 mW/cm^2 for 1000 seconds.

Proper testing of such protocols can take many months or years, as a large number of patients who fit the criteria must be treated, and their long term outcomes assessed, before a good understanding of the success or failure of the treatment programme is reached.

3.8 PDT-Induced Anti-Tumour Immunity

An acute inflammatory response follows the necrosis or apoptosis of the cells due to the cytotoxic ROS produced during PDT. There is some evidence that this mediates an anti-tumour immune response. Roughly, following cell death, antigens (bits of the cell and its contents) are phagocytosed by dendritic cells, which travel to lymph nodes and present the antigens to T lymphocytes, which are activated into effector T cells, which migrate to the tumour and kill the cancerous cells. This is an exciting prospect because, if this effect is found to be significant, the tumour may continue to be destroyed by the immune system even after PDT treatment, and may even leave some longer term protection against similar tumours recurring. It has also been suggested that this sort of approach may be used to develop novel cancer vaccines, by using PDT on cell cultures. However, research is at an early stage with studies in mouse models currently underway.

4 Photothermal: Tissue Heating

Thermal effects are perhaps the most widely encountered form of tissue-laser interaction in clinical practice. They were one of the first to be explored as they do not require the very short laser pulses (ps, fs) which are now available. There are many different and varied medical applications that use a thermal interaction, from vaporization of tumours, to welding gastrointestinal ulcers, and the removal of skin marks such as port wine stain birthmarks or tattoos.

In photochemical effects, such as photodynamic therapy, there is often a specific reaction pathway that leads to tissue damage. With photothermal effects, there is no specific pathway, and the photons may be absorbed by any biomolecule and still lead to a thermal effect. Heat energy is deposited in the tissue by the absorption of light and its subsequent conversion to heat via collisional relaxation. This causes a rise in temperature of the tissue. Then the heat will diffuse through the tissue causing a rise in temperature in the surrounding tissue.

The damage done to the tissue depends on the *temperature* that is reached, and the duration at which it is held at that temperature. First, we look at how we can calculate the temperature rise in the tissue and how the heat diffuses to the surrounding tissue. Then, in the next section, we look at the effect of the raised temperature on different types of tissue - the damage done by the heating. (This is not particular to light-induced heating, but is applicable to thermal tissue damage however the heating is achieved (electrical diathermy, ultrasound heating, etc).)

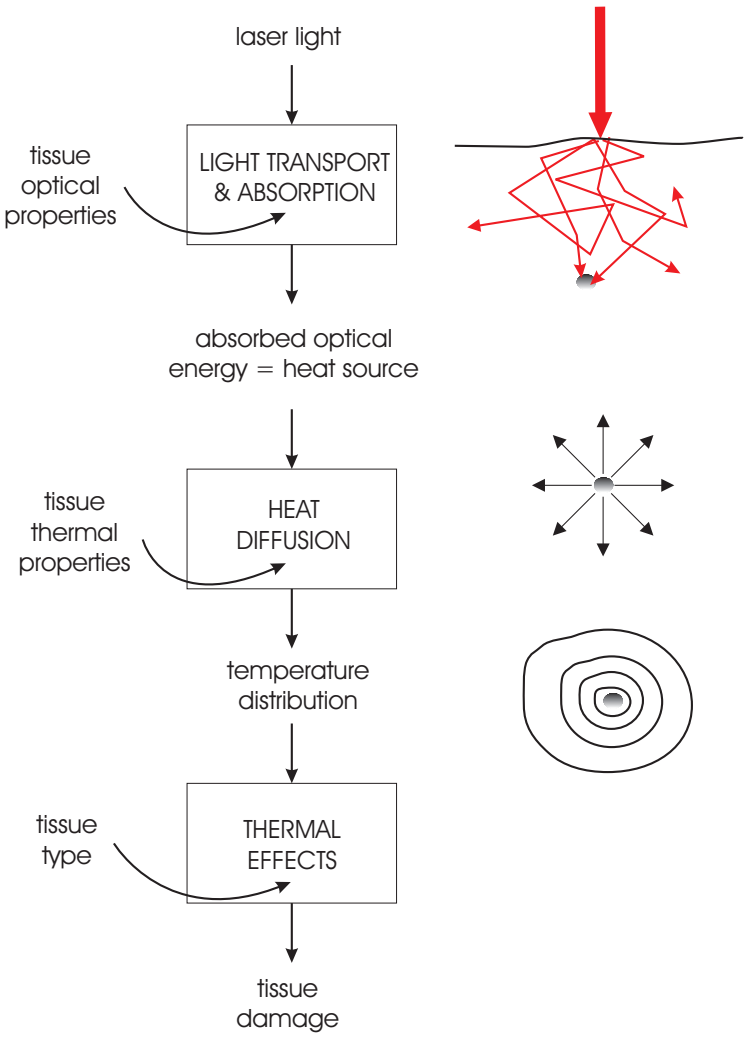


Figure 6: The various aspects involved in thermal interactions of light with tissue.

4.1 Heat Deposition

For the energy in the photons to end up as heat in tissue, two things must happen:

First, absorption. The photon must be absorbed by a molecule, putting that molecule into an excited state. When we are looking at soft tissue and the absorption of infrared light, as we will be in this section, the main chromophores (light absorbers) of interest will be water molecules.

Second, vibrational relaxation. Collisions with other molecules leads to a gradual deactivation of the original molecule (the excited electron moves down the ladder of permitted energy levels) and an increase in the kinetic energy of those it collides with. An increase in the kinetic energies of the molecules is, on a macroscale, an increase in the temperature of the tissue.

4.1.1 Heating with continuous illumination

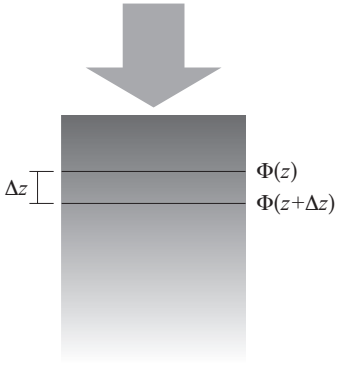
First, consider a continuous (rather than pulsed) laser beam incident on tissue. The light distribution can be described by the fluence rate (or irradiance) Φ (phi) which depends on the position in the tissue $\mathbf{x} = (x, y, z)$. The units of $\Phi(\mathbf{x})$ are Wm^{-2}, so it is a measure of the energy crossing a unit area in a unit time.

During the continuous illumination, the amount of optical energy absorbed per unit volume per unit time is called the *absorbed power density*, $H(\mathbf{x})$ in Wm^{-3}, and is related to the fluence rate Φ by

$$\boxed{H = \mu_a \Phi} \tag{8}$$

where $\mu_a(\mathbf{x})$ is the absorption coefficient, units m^{-1}.

To see where this expression comes from think of the following one-dimensional example: a pure absorber with light incident on it. If the fluence rate at a depth z is written Φ_z, then the difference between the irradiance at depths of z and $z+dz$ is $\Phi(z)-\Phi(z+dz)$. If this difference is non-zero, then some power must have been absorbed. The absorbed power density is the difference in the fluence rate per unit depth, i.e.



$$H = \frac{\Phi(z) - \Phi(z+dz)}{dz} \to -\frac{\partial \Phi}{\partial z} \quad \text{as} \quad dz \to 0 \tag{9}$$

As the fluence rate can be written in this case as $\Phi = \Phi_0 e^{-\mu_a z}$, the absorbed power density can be written as

$$H = -\frac{\partial \Phi}{\partial z} = \mu_a \Phi_0 e^{-\mu_a z} = \mu_a \Phi \tag{10}$$

In fact, the equation $H = \mu_a \Phi$ is always true however complicated the spatial distributions of the irradiance and the absorption coefficient, and not just in this simple 1D example.

$H(\mathbf{x})$ is the optical energy that is being deposited in the tissue as heat every second. This is the heat source term in the equation describing heat transport discussed in a later section.

In the Figure below, the absorption coefficient distribution on the left and the collimated irradiation shown with the arrow, result in the fluence rate distribution (centre) and absorbed power density (right) as shown. (This was calculated using a finite element model based on the diffusion approximation to the light transport equation.) Notice the absorbed power density is not necessarily greatest where the absorption coefficient or the light is greatest, but where their product is.

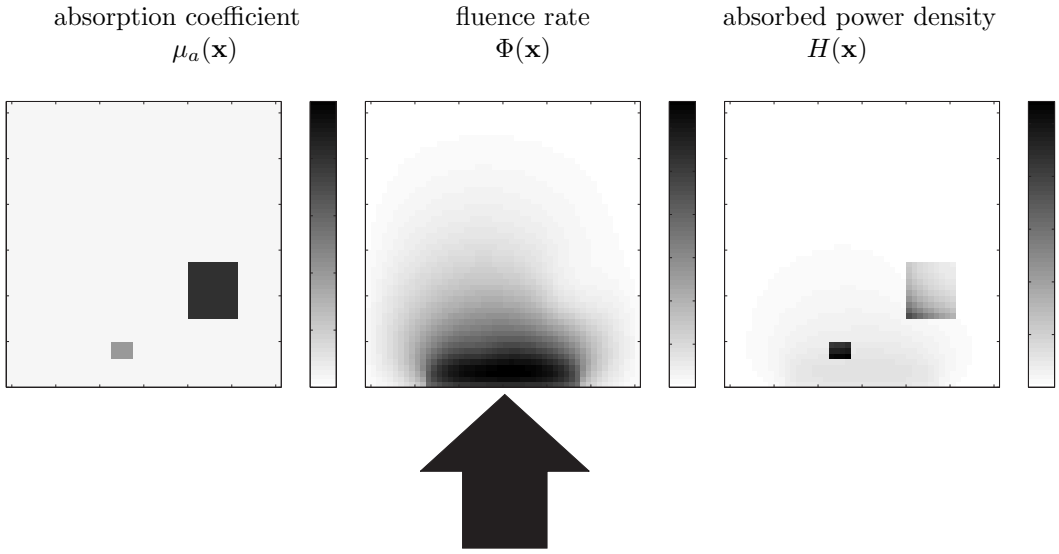


Figure 7: Absorption coefficient, fluence rate and absorbed power density distributions.

4.1.2 Heating with pulsed illumination

Now consider a pulsed laser beam incident on a tissue surface. For the first few moments (of the order of ps) after turning on the laser, the fluence rate within the sample will be changing with time, $\Phi(\mathbf{x}, t)$, before settling down to a steady fluence rate which depends only on space $\Phi(\mathbf{x})$. In practice, as far as photothermal processes are concerned, the fluence rate can be considered to reach this (optically) steady state instantaneously, ie. as soon as the laser is started. The reason for this is that the time it takes the fluence rate to reach a steady state is much shorter than the other timescales of interest, eg. the duration of the laser pulse. This assumption of **instantaneous photon transport** is rarely stated explicitly but often implied. With this assumption, the fluence rate, now a function of time, can be written as

$$\Phi(\mathbf{x}, t) = \Phi(\mathbf{x}) f(t) \quad \text{Wm}^{-2} \tag{11}$$

where $f(t)$ describes the temporal shape of the pulse. ($f(t)$ is dimensionless, ie. it has no units.) The absorbed power density is therefore

$$H(\mathbf{x}, t) = \mu_a(\mathbf{x}) \Phi(\mathbf{x}) f(t) \tag{12}$$

The calculations in the section above considered the heating effect of an unmodulated continuous laser, where photons are continuously being absorbed and so energy absorbed *per second*, or absorbed power, was the quantity of interest. For a pulsed laser, the energy absorbed *per pulse* may be more useful. The absorbed energy density in J/m^3 - the total energy absorbed per unit volume - can be written as

$$E = \int_0^\infty H(\mathbf{x}, t) dt = \mu_a \Phi \int_0^\infty f(t) dt \tag{13}$$

For example, if the laser pulse is a square pulse such that $f(t) = 1$ between 0 and t_p, Fig. 8, then $E = \mu_a \Phi t_p$. We often approximate real pulses as square pulses like this to simplify the calculations.

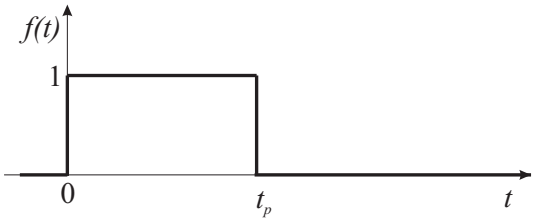


Figure 8: Square pulse.

4.2 Heat Diffusion

4.2.1 Temperature rise

If the tissue does not change phase (boil, for instance) then an increase in the heat energy per unit volume, dE, leads to a rise in temperature dT. The magnitude of this rise can be calculated using the specific heat capacity, C_p, which is defined as the heat energy required to raise 1 kg of substance by 1 K at constant pressure. (The word 'specific' often, but not always, means 'per unit mass'.)

$$C_p \equiv \frac{1}{\rho}\left(\frac{\partial E}{\partial T}\right)_p \tag{14}$$

A heat increase dE (energy per unit volume) and the resulting temperature rise dT are therefore related by

$$dE = \rho C_p dT \tag{15}$$

where ρ is the mass density, kgm^{-3}, and C is the specific heat capacity, $\mathrm{Jkg}^{-1}\mathrm{K}^{-1}$. For water $C_p = 4350\ \mathrm{Jkg}^{-1}\mathrm{K}^{-1}$, but for tissue a better approximation is given by

$$C_p = 1550 + 2800\left(\frac{\rho_w}{\rho}\right) \tag{16}$$

where ρ_w is the density of water and ρ the tissue density. (For liquid water, the difference between the constant-volume and constant-pressure heat capacities, C_v and C_p, is small because of its low thermal expansivity, so sometimes it is not specified which is being used.)

4.2.2 Thermal conductivity

The deposited heat does not remain in the same place for long, but spreads into the surrounding tissue. We therefore need something to describe the flow of heat through the tissue. The heat flux vector, $\mathbf{q} = (q_x, q_y, q_z)$ with units of Wm^{-2} is the amount of heat energy that flows through a unit surface perpendicular to the vector $\mathbf{q}$ in unit time. It is found empirically that the heat flux $\mathbf{q}$ is proportional to the gradient of the temperature, given by the vector ∇T

$$\nabla T \equiv \left(\frac{\partial T}{\partial x}, \frac{\partial T}{\partial y}, \frac{\partial T}{\partial z}\right) \tag{17}$$

We can write that the heat flux and temperature gradient are related by

$$\mathbf{q} = -\kappa \nabla T \tag{18}$$

where the constant κ (kappa) is called the thermal conductivity and has units of $\mathrm{Wm}^{-1}\mathrm{K}^{-1}$. For soft tissue, this can be approximated by

$$\kappa = 0.06 + 0.57\left(\frac{\rho_w}{\rho}\right) \tag{19}$$

Eq. (18) is called Fourier's law of conduction, among other things. Note that if the temperature is the same everywhere, $\nabla T = 0$, no heat flows anywhere, $\mathbf{q} = 0$. Also see that this law says that the heat will flow in the opposite direction to the temperature gradient, ie. downhill, from hot to cold. This is what we would expect from the second law of thermodynamics (and everyday experience).

4.2.3 Heat diffusion equation

It is the temperature distribution that we are interested in, not the heat flux, as the tissue damage is related to the temperature. What we really want is an equation that describes how the *temperature* spreads out in space as time goes on.

To find another equation for $\mathbf{q}$ that we can substitute into Eq. (18), we will use the idea that energy is always conserved. Consider a small, imaginary cube of tissue centred at $\mathbf{x}$ and with sides of length dx, dy and dz. By the conservation of energy, the net amount of heat flowing into that cube per unit volume and per unit time must be equal to the increase in heat energy within the cube, per unit volume and time.

The increase in heat energy within the cube per unit volume and time, ie. the rate of change of heat energy per unit volume, is related to the rate of change of the temperature, $\partial T/\partial t$, via the specific heat capacity C ($\mathrm{Jkg^{-1}K^{-1}}$) and the mass density ρ (rho, $\mathrm{kgm^{-3}}$):

$$\text{increase in energy in cube } = \rho C_p \frac{\partial T}{\partial t} \tag{20}$$

There are two contributions to the net amount of heat entering the cube per unit volume and time. The first is from sources of heat within the cube, eg. the heat source term we saw above, H, due to the absorption of photons. The second is the net amount of heat energy flowing into the cube from the adjacent tissue, described by

$$\text{net heat energy entering cube } = -\nabla \cdot \mathbf{q} + H \tag{21}$$

where $\nabla \cdot \mathbf{q}$ is the divergence of the heat flux vector $\mathbf{q}$.

What is the physical meaning of the divergence? We can understand this better by considering just one direction, say the x-direction. See Fig. 9. The amount of heat flowing in through the lefthand-side of the cube per unit time is $q_x(\mathbf{x} - dx/2)dydz$ and the amount of heat flowing in through the righthand-side of the cube per unit time is $-q_x(\mathbf{x} + dx/2)dydz$. Because $q_x dydz$ is in units of W, the net amount of heat energy *per unit volume* and per unit time entering the cube in the x-direction is $(q_x(\mathbf{x} - dx/2) - q_x(\mathbf{x} + dx/2))/dx$. As $dx \to 0$,

$$\frac{q_x(\mathbf{x} - dx/2) - q_x(\mathbf{x} + dx/2)}{dx} \to -\frac{\partial q_x(\mathbf{x})}{dx} \tag{22}$$

Adding in the contributions from the y and z directions gives the negative of the divergence of $\mathbf{q}$, written $\nabla \cdot \mathbf{q}$, as

$$\left(\frac{\partial q_x}{dx} + \frac{\partial q_y}{dy} + \frac{\partial q_z}{dz}\right) \equiv \nabla \cdot \mathbf{q} \tag{23}$$

Now we are ready to equate the increase in energy in the cube , Eq. (20), with the divergence of the net energy entering the cube, Eq. (21):

$$\rho C_p \frac{\partial T}{\partial t} = -\nabla \cdot \mathbf{q} + H \tag{24}$$

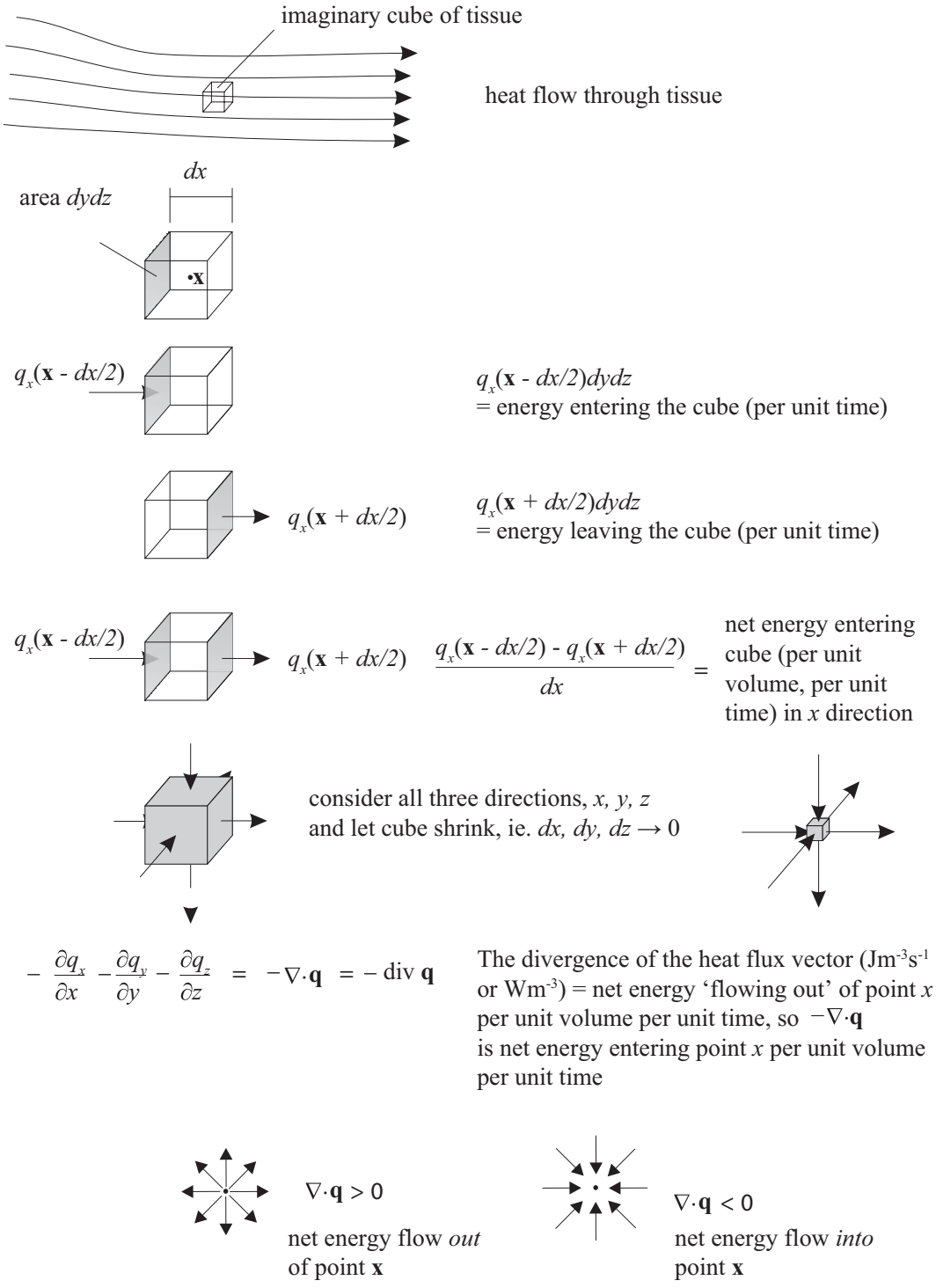


Figure 9: Physical meaning of the divergence of the heat flux vector.

By combining equations (18) and (24) we can derive an equation for dependence of the temperature T on the source term H

$$\rho C_p \frac{\partial T}{\partial t} - \nabla \cdot (\kappa \nabla T) = H \tag{25}$$

which, when κ is uniform throughout the tissue becomes

$$\boxed{\rho C_p \frac{\partial T}{\partial t} - \kappa \nabla^2 T = H} \tag{26}$$

This partial differential equation (pde) is called the (heat) diffusion equation, and as well describing thermal diffusion, has been used to model the diffusion of particles in solution, people in crowds, and so on. Sometimes this equation is written as

$$\frac{\partial T}{\partial t} - D\nabla^2 T = \frac{H}{\rho C_p} \tag{27}$$

where $D \equiv \kappa / \rho C_p$ is called the thermal diffusivity.

Looking at Eq. 26 we can see that, with everything else held constant, when the source term, H, is positive, $\partial T/\partial t$ is positive, so the temperature tends to increase. Similarly, where the 'curvature' of the temperature $\nabla^2 T$ is positive, the temperature will tend to increase, and where $\nabla^2 T$ is negative, the temperture will tend to decrease (Fig. 10).

4.2.4 Pennes' bioheat equation

As well as thermal *conduction*, which is what diffusion is, heat can be transferred by *radiation* and *convection*. These can be neglected in laser applications with short duration pulses, but where blood flow or perfusion through tissues is significant, such as in laser angioplasty, the heat carried away by the blood may have to be taken into account by introducing a loss term into Eq. (26). This additional term was first introduced by Pennes in 1948 and is written

$$H_p = P \rho_b C_b (T - T_b) \tag{28}$$

where T is the tissue temperature, T_b, C_b and ρ_b are the temperature, specific heat capacity, and density of blood, P is the blood perfusion which is the volume of blood flowing through a unit volume of tissue per second. The bioheat equation is then written as

$$\rho C_p \frac{\partial T}{\partial t} - \kappa \nabla^2 T = H - H_p \tag{29}$$

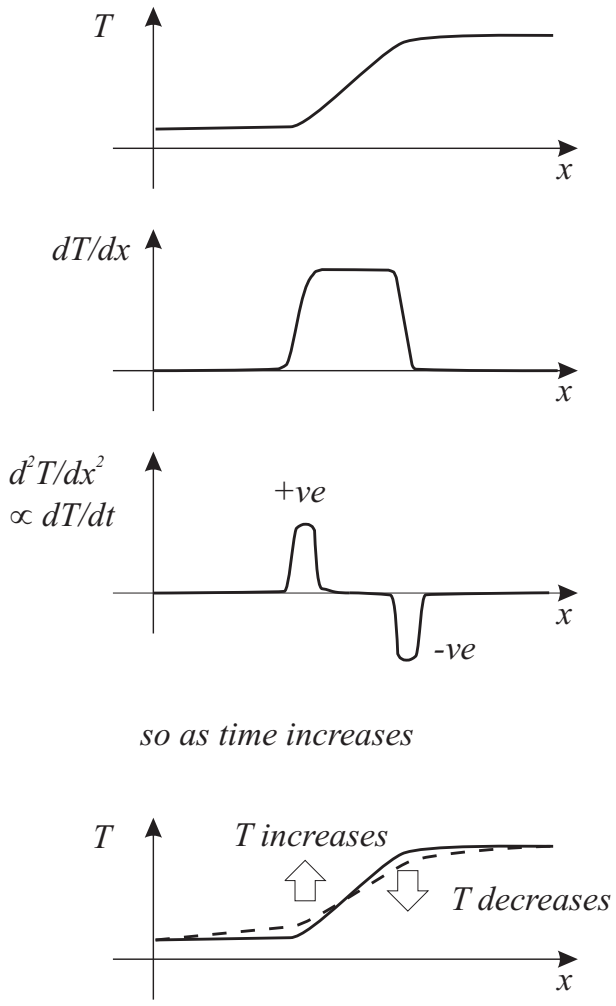


Figure 10: In a region with negative 'curvature' of the temperature, the temperature will decrease.

4.3 Solutions to the Heat Equation

Here we are concerned with finding a solution to the heat diffusion equation

$$\frac{\partial T}{\partial t} - D\nabla^2 T = \hat{H} \tag{30}$$

where $D \equiv \kappa/\rho C_p$ is the thermal diffusivity and the source term $\hat{H} = H/(\rho C_p)$ arises from the absorption of laser light energy and its subsequent conversion to heat. [2]

Numerical methods such as the finite-difference or finite-element methods can be used to solve the diffusion equation under very general conditions. Many numerical analysis packages have a diffusion equation solver built in, making it (relatively) easy for the user to predict what will happen in any particular case.

However, it is very important to have a feel for what sort of solution you expect in a given situation, even if you can only put imprecise numbers on it, for several reasons. For a start, it is important so that you can see instantly when numerical calculations look completely wrong. This sort of physical intuition or insight is also important in trying to solve a problem in which, say, thermal diffusion might or might not be important and you need to think of experiments to find out whether it is. Solutions to simple cases (simple geometries, temperature-independent and uniform material properties for example) give us a guide as to how heat behaves, and can even be applied directly to a number of practical cases.

Continuous illumination: thermally steady state When a sample is being continuously illuminated by a constant power laser beam, and has been for some time so the temperature of the sample has settled down to a steady state everywhere, ie. is not changing with time, then it is in a (thermally) steady state. In this case, T is not changing with time so $\partial T/\partial t = 0$ and the diffusion equation reduces to Poisson's equation,

$$\nabla^2 T = -\frac{\hat{H}}{D}. \tag{31}$$

Poisson's equation, like the diffusion equation, has been studied extensively, but no more details will be given here.

[2] The heat diffusion equation is unrealistic in one sense: acccording to it, if heat is deposited at a point $\mathbf{x}'$ then the temperature at another point $\mathbf{x}$ will begin to rise *immediately following the deposition of the pulse.* There is no time delay for the heat to reach $\mathbf{x}$, it just starts getting hotter immediately. This equation is therefore non-causal (unlike real life) and so sometimes extra care is necessary when interpreting it. Having said that, it is rarely a problem in practice and the heat diffusion equation is the standard way to describe heat transport.

4.3.1 Green's functions: fundamental solutions

Green's functions are fundamental solutions to partial differential equations. For the heat diffusion equation, the Green's function is the solution when the source term $\hat{H}(\mathbf{x}, t)$ is a point source with an infinitesimally-short duration. Such a source can be represented mathematically by using delta-functions $\delta(\mathbf{x} - \mathbf{x}')\delta(t - t')$. For instance, in the equation

$$\frac{\partial g}{\partial t} - D\nabla^2 g = \delta(\mathbf{x} - \mathbf{x}')\delta(t - t'), \tag{32}$$

the heat is deposited instantaneously at time $t = t'$ at point $\mathbf{x} = \mathbf{x}'$. The Green's function $g(\mathbf{x}, t; x', t')$ is the solution to this equation; it describes how the temperature distribution varies over time t and space $\mathbf{x}$ following the instantaneous deposition of heat at point $\mathbf{x}'$ at time t'.

The symbol G is often used for a Green's function, but it is still describing the temperature. If the source really can be described as a point source with a short pulse duration, then the Green's function gives the temperature field. It just is the solution for the temperature, without any further calculation. However, Green's functions are more useful than that because you can treat distributed (non-point-like) sources like $\hat{H}(\mathbf{x})$ as if they are made up of lots of point sources, and so just add (integrate) together the solutions. For instance, the solution to Eq. 30 can be written in terms of the Green's function g, the solution to Eq. 32 as

$$T(\mathbf{x}, t) = \int_0^t \int_V \hat{H}(\mathbf{x}', t')g(\mathbf{x}, t; x', t')d\mathbf{x}'dt' \tag{33}$$

The inner integral sums over the source positions $\mathbf{x}'$ and the outer over all times up to the time of interest, t.

Solution for a point source in 3D. The Green's function solution to Eq. (32) for a point source in an infinite, 3D piece of homogeneous tissue is

$$g(\mathbf{x}, t; x', t') = \left(\frac{1}{8(\pi D(t - t')^{3/2}}\right) \exp\left(-\frac{|\mathbf{x} - \mathbf{x}'|^2}{4D(t - t')}\right) \tag{34}$$

The solution for any source distribution can be built using this Green's function. A trivial but useful example: if E Joules of optical energy are deposited instantaneously at point $\mathbf{x}' = 0$ and time $t' = 0$, then the temperature distribution will spread out according to

$$T(\mathbf{x}, t) = \frac{E}{\rho C_p}\left(\frac{1}{8(\pi Dt)^{3/2}}\right) \exp\left(-\frac{|\mathbf{x}|^2}{4Dt}\right) \tag{35}$$

This is illustrated in Fig. 11.

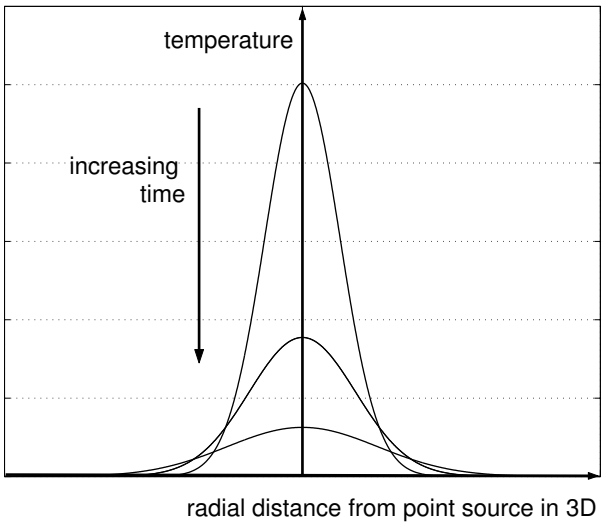


Figure 11: Solutions to the heat diffusion equation, at increasing values of time, for an instantaneous point source in 3D. The solutions are radially symmetric.

Solution for a line source in cylindrical coordinates. If the heat source can be modelled as a line, eg. a thin blood vessel heated by absorbing light, then the temperature a radial distance r from the line is

$$T(r,t) = \frac{E}{\rho C_p}\left(\frac{1}{4\pi Dt}\right)\exp\left(-\frac{r^2}{4Dt}\right) \tag{36}$$

Solution for a plane source (1D). Imagine a 3D block of homogeneous tissue uniformly illuminated from one side so that the light and temperature distributions depend only upon depth, z. This is a 1D case in the sense that only one spatial variable (depth) is needed to describe the distributions. In this case the Green's function is

$$T(z,t) = \frac{E}{\rho C_p}\left(\frac{1}{2\sqrt{\pi Dt}}\right)\exp\left(-\frac{z^2}{4Dt}\right) \tag{37}$$

4.3.2 Thermal penetration depth

The solution for the temperature in Eq. (37) contained the Gaussian $\exp(-z^2/4Dt)$. Because, in this example, the heat energy was all deposited at $z = 0$, the temperature at $z = 0$ will always be greater than the temperature elsewhere. In fact, at time t, the temperature at depth $z = \sqrt{4Dt}$ will be $1/e \approx 0.37$ of the temperature at $z = 0$. This is true for all times t, so the distance $\sqrt{4Dt}$ is a characteristic length called the *thermal penetration depth*. This gives us a rough idea of how deeply the heat has penetrated into the tissue t seconds following an instantaneous heating pulse.

The thermal penetration depth, z_{th} is defined as

$$z_{th} = \sqrt{4Dt} \tag{38}$$

Notice this is not a linear relationship between penetration depth and time. In tissue, the diffusivity $D \approx 114 \times 10^{-9}$ $\mathrm{m^2 s^{-1}}$ so heat diffuses to about 0.7 μm in 1 μs, to 2.1 μm in 10 μs, to 7 μm in 100 μs, to 21 μm in 1 ms, and so on.

4.3.3 Thermal relaxation time

In a similar way, the *thermal relaxation time* tells us how long it will take for the temperature at depth z to reach $1/e$ of the temperature at $z = 0$. In other words, it gives an indication of how long it takes the heat to travel a distance z.

The thermal relaxation time gives an indication of how quickly an object loses its heat.

The relaxation time can help us decide whether it is important to include thermal diffusion in our calculations or not. For processes or events that happen much more quickly than the relaxation time, we can ignore thermal diffusion. The thermal relaxation time for the 1D planar case is approximately

$$t_{\text{planar}} \approx \frac{z_0^2}{4D} \tag{39}$$

For example, when light is incident on a *perfect absorber*, the heat deposited falls off exponentially with depth, $\exp(-\mu_a z)$ so $1/\mu_a$ is a characteristic distance of the heated region. The thermal relaxation time in this case is

$$t_{\text{absorber}} \approx \frac{1}{4D\mu_a^2}. \tag{40}$$

The approximate signs $\approx$ were used above because Eq. 37 has a $1/\sqrt{t}$ term as well as the Gaussian $\exp(-z^2/4Dt)$, so in fact the temperature will reach $1/e$ slightly more quickly than this expression suggests. This formula for the relaxation time is for a slab - the 1D case. The equivalent expressions for cylinders and spheres are

$$\tau_{cylind} \approx \frac{d^2}{16D}, \qquad \tau_{sphere} \approx \frac{d^2}{24D}. \tag{41}$$

where d is the diameter.

• **Simple derivation of thermal relaxation time in 1D** Consider a one-dimensional block of tissue that is at a temperature ΔT above the surrounding tissue (Fig. 12). To estimate the heat energy contained in the block, in Joules, use the definition of the specific heat capacity: C_p is the energy required to raise 1 kg of material by 1 degree K, so the units are $\mathrm{Jkg^{-1}K^{-1}}$. Multiplying by the density gives ρC_p, with units of $\mathrm{Jm^{-3}K^{-1}}$. Multiplying by temperature gives $\Delta T \rho C_p$, with units of $\mathrm{Jm^{-3}}$. The heat contained in a volume xyz_0 of tissue is therefore

$$\text{heat contained} = (xyz_0)\Delta T \rho C_p \quad \text{Joules} \tag{42}$$

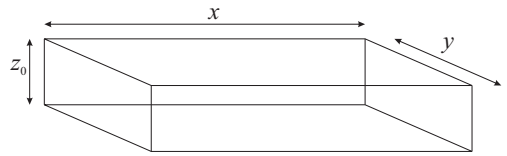


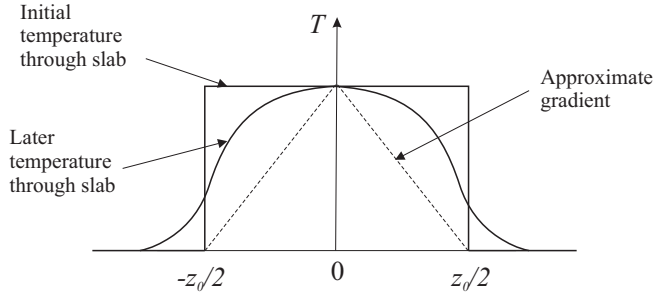
Figure 12: Block of tissue used in estimating the thermal relaxation time.

The amount of heat energy conducted away from the block each second can be estimated by considering the definition of the thermal conductivity: $\kappa \equiv \mathbf{q}/\nabla T$, where $\mathbf{q}$ is the heat energy per unit time crossing a unit area ($\mathrm{Wcm^{-2}}$), and ∇T is the temperature gradient ($\mathrm{Km^{-1}}$). The heat lost per second is therefore (surface area)$\times\kappa\nabla T$. If the block is part of a much larger block of the same thickness z_0 (1D case) then the heat will only be lost from the upper and lower surfaces (any heat leaving the sides of our block will be balanced by heat entering from the adjacent identical block). If we approximate the temperature gradient as $\nabla T \approx \Delta T/(z_0/2)$ (see Figure below) then we can write the heat loss per second as

$$\text{heat loss per second} = (2xy)\kappa\Delta T/(z_0/2) \quad \text{Joules/second} \tag{43}$$

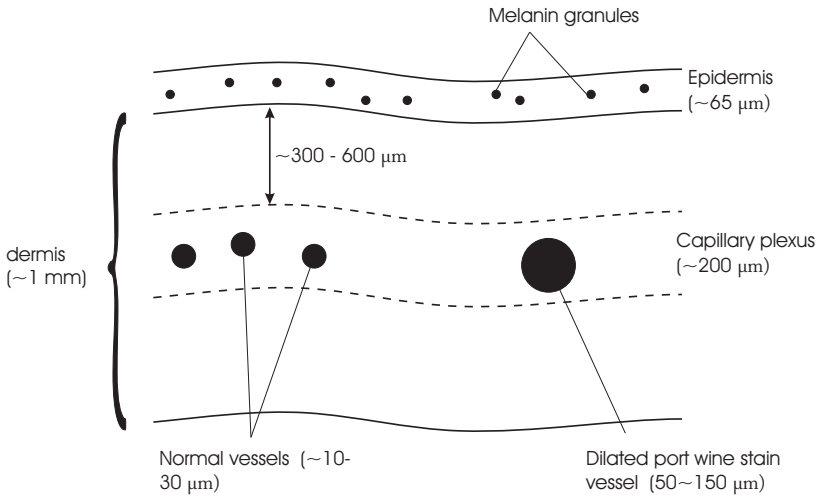
The thermal relaxation time, τ_{planar}, is the ratio of the heat content and the rate of heat loss, so can be estimated as

$$\tau_{planar} \approx \frac{(xyz_0)\Delta T \rho C_p}{(2xy)\kappa\Delta T/(z_0/2)} = \frac{z_0^2}{4D}. \tag{44}$$



4.4 Example: Selective photothermolysis to treat port wine stain

Port wine stain, or *naevi flammei*, is a red and often unsightly birth mark caused by enlarged capillary vessels in the dermis. The capillaries in the dermis are usually $\approx 10 - 30$ μm in diameter; the enlarged vessels can be as large as ≈ 150 μm in diameter.



Two factors allow these vessels to be thermally coagulated without damaging either the surrounding tissue, including the other vessels, their absorption and their size.

First, Selective Photothermolysis This refers to a technique whereby we choose a laser wavelength which is absorbed much more strongly by the blood (in this case the haemoglobin molecules in the blood) than by the surrounding tissue. A dye laser with $\lambda = 577$ nm is a good choice.

Second, Pulse Duration We want to choose a pulse duration long enough that the small vessels will lose their heat during it, but short enough so that the larger vessels won't. In this way, the large vessels can reach a higher temperature than the smaller vessels. As the thermal relaxation time of a cylindrical vessel can be estimated using $\tau_{\text{cylind}} \approx d^2/16D$ and the diffusivity of tissue is $D \approx 114 \times 10^{-9}$ m^{2}s^{-1}:

$\tau_{30} \approx 0.5$ ms and $\tau_{150} \approx 12$ ms

So we choose a laser pulse duration between the two, 0.5 ms $< t_{pulse} <$ 12 ms, erring, for the sake of caution, towards the longer pulse.

Third, Irradiance Now the larger vessel will heat up more than the smaller one, we need to ensure that it will heat up sufficiently to coagulate in the alloted time t_{pulse}. (See Arrhenius equation later on.) We choose the irradiance to ensure enough energy is provided for coagulation.

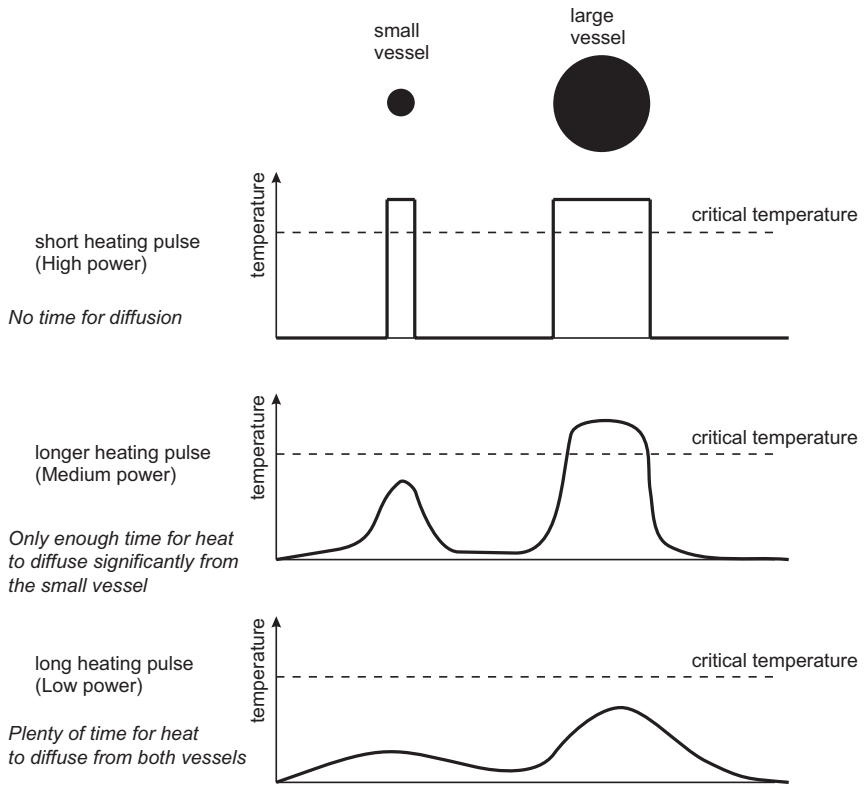


Figure 13: Big things lose their heat more slowly than small things. In these three cases, *the same amount of energy is delivered* to, and absorbed by, the blood vessels. In the top case, the energy is delivered in a very short time (hence a high power pulse) and the heat has no time to diffuse away from the blood vessels of either size, and both are coagulated. In the bottom case, the energy is delivered very slowly (a long, low power pulse) and the heat has time to diffuse away from both the small and large vessels and neither is coagulated. Only in the middle case, where the pulse duration has been chosen so that the heat has time to diffuse away from the small vessel but not from the large vessel, does selective thermal coagulation occur.

4.5 Example: Tattoo removal

Before medical lasers, removing tattoos was a difficult, painful and could be disfiguring, as the only way to remove them was to somehow remove the top layers of skin (abrasively, chemically, surgically,...). Now it is possible to remove tattoos using lasers, although the quality of the outcome varies from person to person. Often complete removal is not possible and some scarring may result. Professional tattoos have proved harder to remove completely than home-made ones, as the ink usually lies in deeper tissue.

• **Laser wavelength** The tattoo ink is a different colour from the skin, so the first thing to do is to choose a laser wavelength that is absorbed by the tattoo ink but not by the skin tissue. If the type of ink used in the tattoo is known, then its absorption spectrum can be measured and the absorption peaks located.

• **Selective photothermolysis** As above with port wine stain treatment, this refers to the selective necrosis of cells based on their colour: ie. using light (photo) to heat cells (thermo) to kill them (lysis). Here, the cells containing the ink particles are lysed and the ink particles themselves are fragmented, so that they are both free and small enough to be removed naturally.

Regret-free tattoos are just becoming available from companies such as Freedom-2. There seem to be two basic ways of making a tattoo removable, but it is too early to say how well they actually work.

• **Microencapsulation** Tiny capsules containing very small ink particles are used to give the tattoo. When it is no longer wanted, a laser is used to rupture the capsules and release the tiny ink particles which are small enough to be cleared away naturally.

• **Colour-change ink** The second proposal is to use an ink that remains permanently in the skin, but which can be made transparent in the visible spectrum by pulsing with a certain wavelength of light. (If this can be reversed by pulsing with a different wavelength of light, then on/off tattoos will be a possibility.)

4.6 Example: Photoacoustic imaging

The two previous examples were both for treatments. Photothermal effects can also be useful for diagnosis. Photoacoustics is a new imaging technique with potential applications in breast imaging, and proven ability to visualis blood vessel microstructure to 1-2 cm deep (which is useful for looking at tumours, where the vasculature is unusual). It is already in use for preclinical research (imaging small animals).

The basic principles are as follows:

- The tissue is illuminated by a wide-beam, short duration, laser pulse, typically from a Q-switched Nd:YAG laser and a few ns long.
- The light is scattered throughout the tissue, and absorbed by chromophores within the tissue, eg. by the heme molecules in hemoglobin in red blood cells.
- The photon energy is converted to heat through vibrational/collisional relaxation leading to a localised temperature and pressure rise. This happens sufficiently fast that the tissue has no time to deform - no time for the density to change. The pressure rise is known as the *initial acoustic pressure distribution*.
- As tissue is elastic, the pressure increase propagates away from the heated region as an acoustic (ultrasonic) pulse and it detected by an array of ultrasound detectors on the surface of the tissue.
- An *image reconstruction algorithm* is used to convert the measured acoustic pressure time series into an image of the initial acoustic pressure distribution, which is the photoacoustic image.

The pulses used to generate photoacoustic waves, and the subsequent acoustic propagation, both occur on a time scale must shorter than thermal diffusion, and so it is usually neglected. As this is the case, the energy is deposited at a rate $H(\mathbf{x}, t) = \mu_a(\mathbf{x})\Phi(\mathbf{x})f(t)$ where $f(t)$ is the temporal shape of the laser pulse (normalised so it has integral 1). The quantity of interest is the total energy delivered during the duration of the pulse, which the *absorbed energy density* given by

$$H(\mathbf{x}) = \int H(\mathbf{x}, t)dt = \mu_a(\mathbf{x})\psi(\mathbf{x}) \tag{45}$$

where $\psi = \int \Phi dt$ is the time integral of the fluence rate, and is known as the *fluence*. The constant which related this absorbed energy density, H, to the initial acoustic pressure distribution, p_0, is a thermodynamic quantity called the Grüneisen parameter, Γ

$$p_0 = \Gamma H. \tag{46}$$

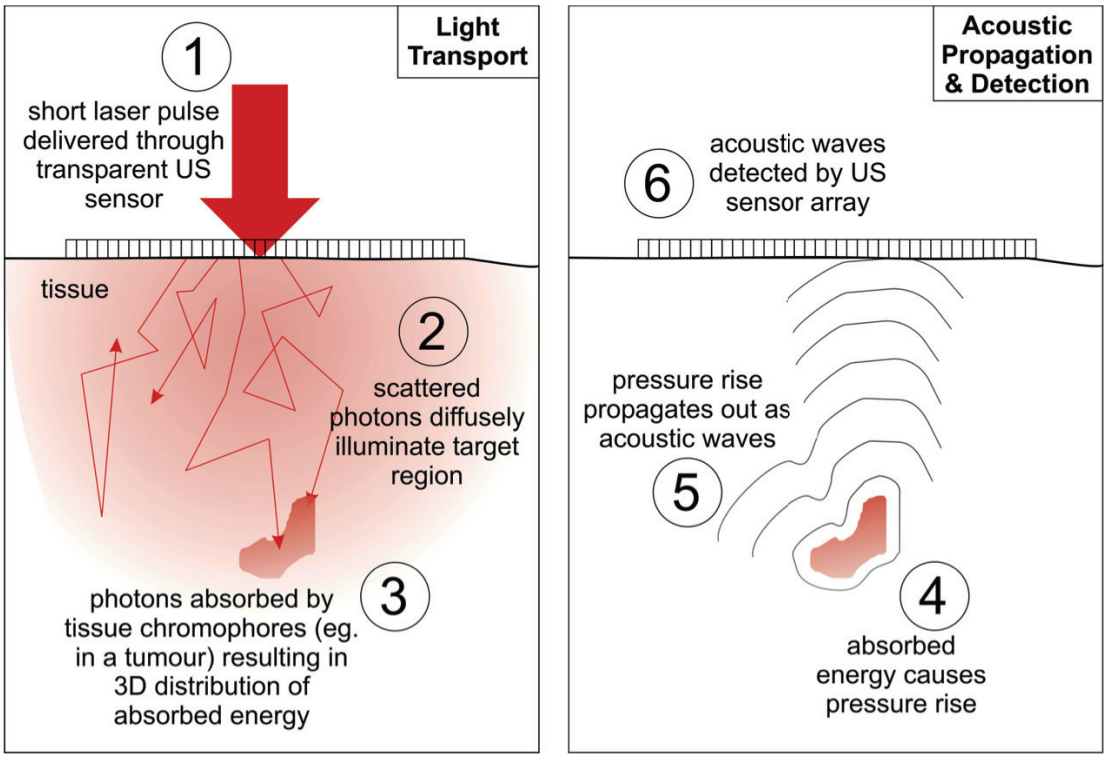


Figure 14: The principles of photoacoustic imaging

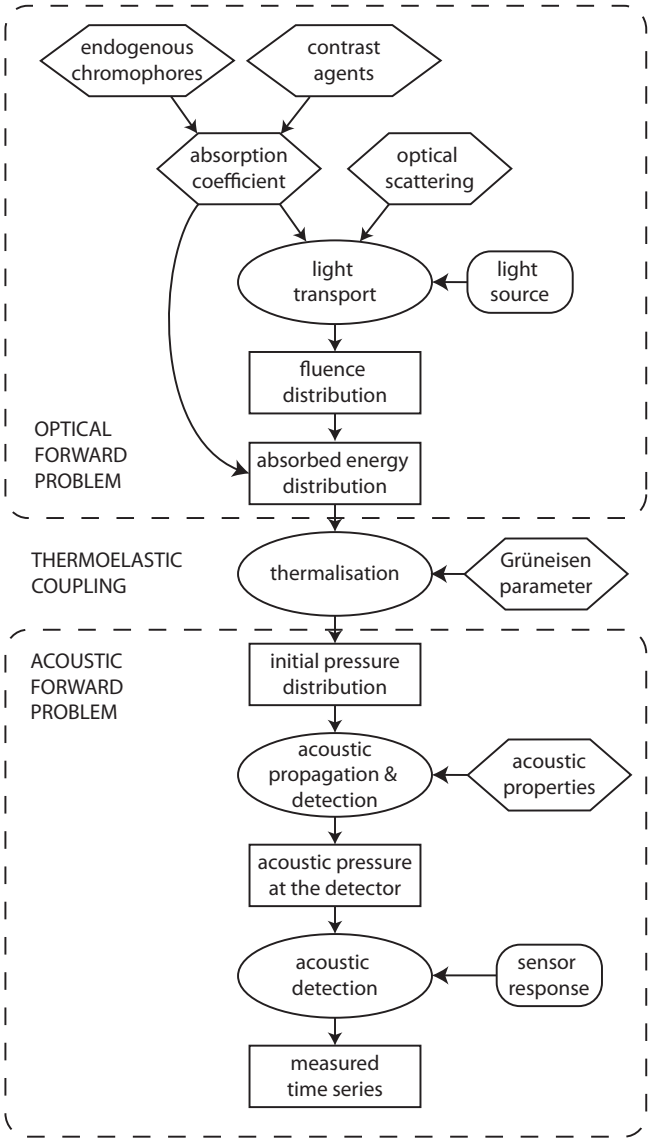


Figure 15: Photoacoustic signal generation

5 Effects of Temperature on Tissue

5.1 Tissue Composition

Looking back to Fig. 6 we can see that to understand how light interacts thermally with tissue, we first need to model the light transport and work out where the light energy is being deposited as heat energy. Second, we need to take into account the fact that soft tissue conducts heat, which we do by solving the heat diffusion equation, or by using the concepts of thermal penetration depth and thermal relaxation time.

Finally, we want to understand the last part of Fig. 6, which is what happens to the tissue once it has reached a certain temperature. To do this we must have an idea of what the tissue is made of. As far as we are concerned in this section, the tissue consists of collagen, water, haemoglobin and perhaps a few other chromophores, such as melanin. This is a gross oversimplification of tissue biology, but is a useful reduction in complexity which can help us understand more easily some of the thermal effects taking place in the tissue.

• Cells: this is the part of this tissue that biologists have conventionally concentrated on. For our purposes cells are often treated as water-filled, although the proteins in certain cells can be crucial to some applications (such as the haemoglobin in red blood cells is to port wine stain treatment).

• Extracellular matrix (ECM): this is a fibrous scaffold among which the cells nestle, and which gives tissue most of its stiffness and structure. It is made from collagen and elastin and other glycoproteins and proteoglycans. The ratio of the amount of ECM to number of cells varies widely depending on the type of tissue. Liver and muscle, for instance, are low in ECM, whereas bone, tendon and the retina are largely ECM. Fig. 16 shows images of extracellular matrix from artifically engineered tissue and *ex vivo* tissue. The collagen in ECM is of interest when considering thermal effects because it breaks down at temperatures well below 100°C. (The chef Heston Blumenthal uses light to soften the collagen in steaks before cooking them, to make them more tender.)

Fig. 17 shows the absorption coefficients of some of the main, optically-relevant, tissue constituents. Notice where the water absorption drops very low, allowing the light in this wavelength range to penetrate deep into the tissue.

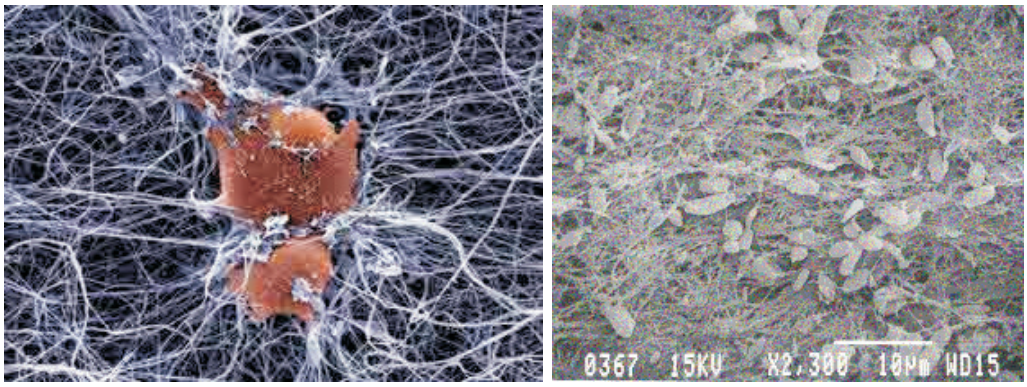


Figure 16: Cells held in extracellular matrix, in varying ratios of cell/matrix

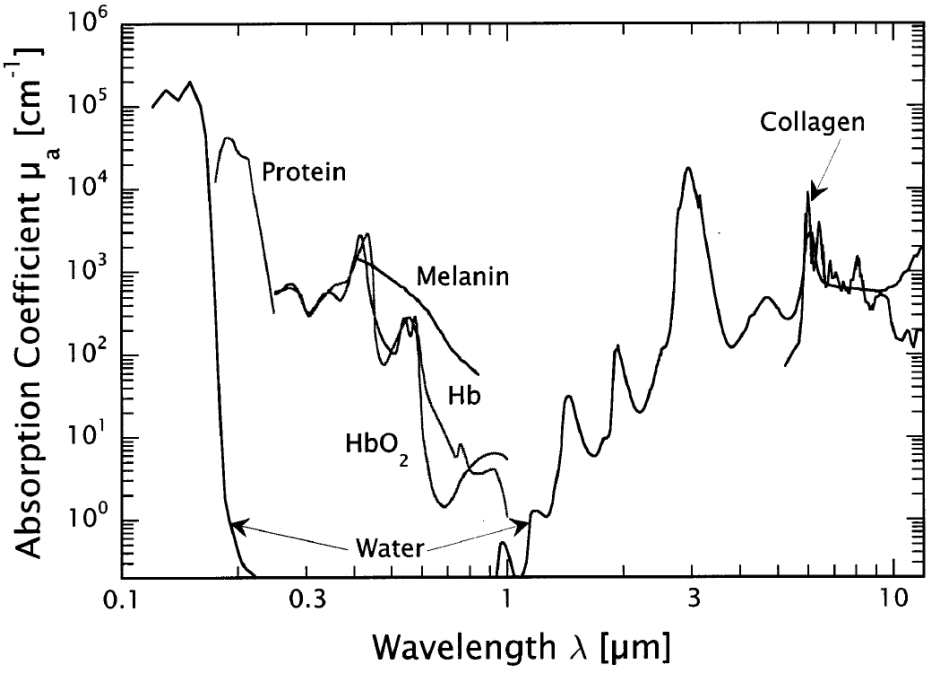


Figure 17: Absorption coefficient spectra for various tissue constituents. Note the peak in the haemoglobin (Hb and $Hb0_2$) absorption at 577 nm used in port wine stain treatment.

5.2 Temperature Effects

• $\sim 37°$C is normal body temperature, and for the first 1.5°C or so of heating few irreversible changes occur.

• At $\sim 41°$C a number of effects, covered with the blanket term *hyperthermia*, begin. Cell proteins - both membrane and cytoplasmic proteins - start to undergo conformational (shape) changes. They change conformation because the hydrogen bonds keeping them in their native state are broken by the increasingly violent vibrations of the molecule as the temperature increases. When a protein molecule changes shape it can often no longer fulfill its function within the cell. For instance, when enzymes, whose catalytic functions depend crucially on their shape, begin to deform, reaction rates within cells slow down. Even at small increases in temperature some cells will die because of these effects. The rate at which cells necrose (or apoptose) increases with the temperature.

• From $\sim 45°$C the collagen fibres forming the ECM begin to shrink as the collagen's trihelical structure breaks apart. The optical scattering in the tissue increases - it whitens (think of frying chicken breast), then the collagen softens and gelatinises. (Gelatin is just tangled, random coils of collagen.) Tissue starts to coagulate, and blood clots form.

• At 100°C the water in the cells and extracellular fluid boils. The huge volume expansion as the water changes phase (vapourises) can lead to tissue being expelled from the skin surface.

• Once all the water has boiled off, the remaining organic material may char (carbonise, blacken). At very high temperatures it will eventually evaporate.

5.3 Hyperthermia: Damage Integral

Hyperthermia is a general term used to describe tissue damage due to heating to temperatures below 100°C. This might be collagen gelatinisation and subsequent tissue coagulation, or the denaturation of cell membranes or cytoplasmic proteins leading to necrotic or apoptotic cell death.

Irreversible tissue damage due to hyperthermia begins around 41° as proteins begin to denature, and it depends on the time at which the tissue is held at a given temperature. eg. 63% of epidermal tissue will die if it is held at 45°C for 9 hours, but the same amount of damage will occur in about 1 second at 60°C.

The amount of damage that is done to the tissue eg. the proportion of the tissue damaged, or of cells killed, or of molecules denatured, can be modelled using a 'damage integral'. The following model gives us a way to estimate the proportion of tissue damaged after a given time.

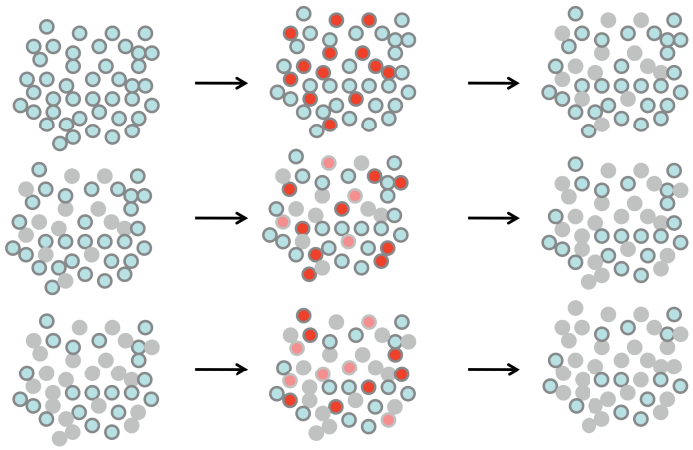


Figure 18: If a fixed proportion of healthy cells are killed per unit time, then the overall decrease in the number of healthy cells will be exponential.

Let C_0 be the initial amount of undamaged tissue and $C(t)$ the amount of undamaged tissue remaining after time t. (C could also represent the number of intact cells or perhaps the concentration of biomolecules still functioning correctly.) If we assume that, at a given temperature, a *fixed fraction* of the healthy tissue will be damaged per unit time then we can

write that the rate of change of C with time is proportional to C, i.e.

$$\frac{dC}{dt} = -kC \tag{47}$$

In other words, whatever the amount of undamaged tissue that actually remains at any instant, a proportion k of it will be damaged in one second. k, with units of s^{-1}, is a rate constant and depends on the temperature and type of tissue.

If k is constant with respect to time, then the amount of undamaged tissue decreases exponentially because the solution to Eq. (47) is

$$C(t) = C_0 e^{-kt} \tag{48}$$

However, k usually depends on the time, because the temperature is often changing with time, eg. during a laser pulse, so it is necessary to integrate Eq. 47 over time. Integrating from time 0 to t gives

$$\int_{C(0)}^{C(t)} \frac{1}{C} dC = -\int_0^t k dt' \tag{49}$$

$$\ln\left(\frac{C(t)}{C_0}\right) = -\int_0^t k dt' \equiv -\Omega \tag{50}$$

This is the 'damage integral' referred to above. The proportion of undamaged tissue can now be written as

$$\frac{C(t)}{C_0} = e^{-\Omega(t)}. \tag{51}$$

How much time must elapse before the tissue is considered non-viable? Because the damage process is modelled as an exponential decay, we would have to wait an infinite time for *all* the tissue to be damaged, so we need another end-point. From Eq. (51) we can see that when the damage integral $\Omega = 1$, only 37% of the undamaged tissue remains. This somewhat arbitrary point is often taken as indicative of irreversible tissue damage. The assumption is that once 63% of the tissue is dead, the tissue as a whole is no longer viable.

5.3.1 Denaturation Kinetics and Arrhenius' Equation

Sub-boiling thermal tissue damage is caused by the denaturation (changing shape, structure) of the collagen, proteins, and the other biomoleules making up the tissue. Consider a biomolecule in its native, 'healthy' state, its usual configuration at 37 °C. To change shape, ie. to break certain bonds and form others and thereby move to another state, will require energy to 'shake the molecule apart'. This is called the 'activation energy', E_a, and will be different for different molecules and different denaturation pathways.

Figure 19 shows a typical energy curve for this transition from native state to denatured state. As all molecules are in constant motion, a molecule in the native state might, by chance, overcome the activation barrier and reach the denatured state. The likelihood of this increases as the temperature increases, as the molecule is vibrating more vigourously, and

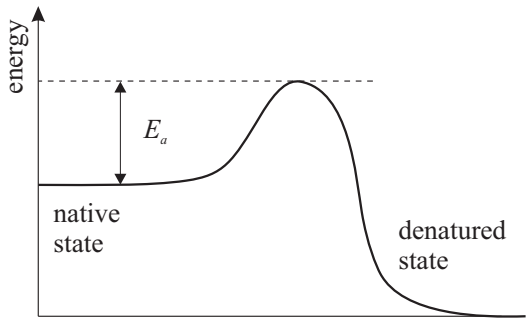


Figure 19: For a molecule to denature an energy barrier must be overcome.

so the rate at which molecules become denatured increases too. Arrhenius noticed that this temperature dependence of the rate constant often took the form

$$k = Ae^{-E_a/RT} \tag{52}$$

where $R = 8.31$ JK^{-1}mol^{-1} is the gas constant, E_a is the activation energy *per mole* and A, with units s^{-1}, is called the pre-exponential factor or prefactor. Eq. (52) is called Arrhenius' equation, and the prefactor A and activation energy E_a are together known as Arrhenius' parameters.

Clearly, the activation energy required to denature a molecule will vary depending on the molecule, and molecular states, in question. The rate at which molecules are denatured, k, will also depend on the environment in which the molecule finds itself, ie. on the tissue type; the prefactor A accounts for this.

5.3.2 Arrhenius' Parameters

By measuring k, the rate of thermal damage to tissue, as a function of temperature, a pair or Arrhenius parameters, E_a and A, may be obtained. Values for A and E_a have been estimated for various tissue types, and are shown in the table below. The reason for the differences between the estimates for nominally the same tissue types is due to differences in the composition of the samples used, and in the measures used to determine the extent of the tissue damage. Several different indicators of damage have been used in obtaining these parameters, including whitening due to an increase in the optical scattering (think of a frying egg), coagulation, contraction, and changes in birefringence.

tissue type	A (s^{-1})	E_a (MJ/mol)
aorta	5.6×10^{63}	0.43
retina	1.0×10^{44}	0.293
	3.1×10^{99}	0.628
	4.32×10^{64}	0.416
	9.39×10^{104}	0.665
skin	3.1×10^{98}	0.628
	1.8×10^{51}	0.327
	2.2×10^{124}	0.783
albumen	3.8×10^{57}	0.385
dog prostate	3.2×10^{8}	0.072
	9.0×10^{12}	0.101
	2.1×10^{27}	0.187
rat liver	2.1×10^{33}	0.222
dog heart	2.9×10^{39}	0.260
pig liver	5.5×10^{41}	0.277
mouse dermis	7.3×10^{64}	0.425
rat tail	1.8×10^{56}	0.368
pig kidney	1.5×10^{60}	0.400
	3.3×10^{38}	0.257

Recall that the damage integral Ω is defined as

$$\Omega \equiv \int_0^t k(t')dt'. \tag{53}$$

Setting $\Omega = 1$ and using Arrhenius' equation, Eq. (52), to describe k, we can write

$$\int_0^{t_n} A \exp\left(-\frac{\Delta E_a}{RT(t')}\right) dt' = 1 \tag{54}$$

In general, this must be solved numerically to obtain t_n, time to necrosis, as a function of position in the tissue. We can simplify, though, when the tissue is kept at a constant temperature, for then the integrand is independent of t, and Eq. (54) becomes

$$t_n A \exp\left(-\frac{\Delta E_a}{RT}\right) = 1. \tag{55}$$

Taking logs turns this into an equation of a straight line. Taking natural logs gives

$$\ln(t_n) = \left(\frac{\Delta E_a}{RT}\right) - \ln(A) \tag{56}$$

or taking logs to base 10,

$$\log_{10}(t_n) = \log_{10}(e)\left(\frac{\Delta E_a}{RT}\right) - \log_{10}(A). \tag{57}$$

This equation tells us how long it takes, t_n seconds, for a tissue, characterised by Arrhenius' parameters A and E_a, to become significantly damaged if it is held at temperature T K. Eq. (57) is plotted in Fig. 20, using a few values of A and ΔE from the table above. According to this model, at 60°C skin coagulates in about $10^0 = 1$ s, but aorta takes 10^4 s, almost three hours.

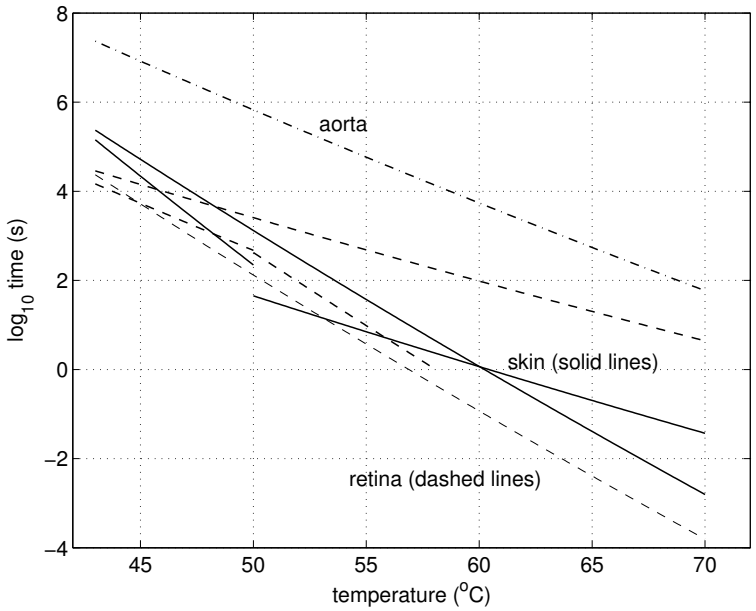
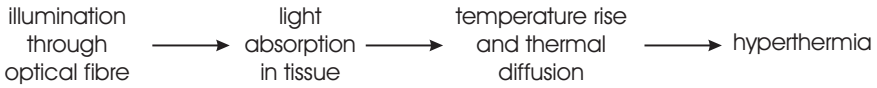


Figure 20: The time is takes different tissue to be significantly damaged when held at constant temperature, according to the Arrhenius' model of damage due to hyperthermia. For instance, skin coagulates in 1 second at 60°C.

5.4 Example: Laser-induced interstitial thermotherapy

Laser-induced interstitial thermotherapy (LITT), also sometimes called interstitial laser photocoagulation (ILP), uses the fact that tissue can be killed at relatively low temperatures to treat tumours and other diseases where tissue destruction is part of the solution.



LITT provides an example of where models of light transport, heat diffusion and thermal damage must be used to predict the damage to tissue following a laser pulse. If an Nd:YAG laser with a wavelength of 1064 nm is used to treat a liver tumour using LITT, how do we calculate how much of the tissue has been killed after a certain time?

- **Light Modelling** First we determine the optical properties of the liver at 1064 nm, ie. the scattering and absorption coefficients μ_s' and μ_a, and model the light transport in the liver - perhaps using a Monte Carlo method - to obtain an estimate of the fluence rate $\Phi(\mathbf{x})$. The absorbed power density in the liver can be calculated from $H = \mu_a \Phi$.
- **Heat Diffusion** The absorbed power density, H, is the source in the heat diffusion equation. We can therefore calculate, using a numerical model of the heat diffusion equation, how the distribution of the temperature $T(\mathbf{x}, t)$ will change with time t throughout the tissue. T will continue to change with time after the pulse has stopped as the heat spreads out, so the whole temperature history of the tissue must be included in the damage integral.
- **Damage Integral** $\Omega = 1$ is our criterion for total damage, so numerically calculating

$$\Omega(\mathbf{x}) = \int_0^\infty A \exp\left(-\frac{E_a}{RT(\mathbf{x}, t)}\right) dt \tag{58}$$

  will indicate in which regions $\Omega(\mathbf{x}) > 1$, and so in which regions tissue will be necrosed. It is therefore necessary to choose the light source positions and duration of the pulse such that $\Omega(\mathbf{x}) > 1$ in the regions of unwanted (diseased) tissue and, ideally, $\Omega(\mathbf{x}) \ll 1$ in healthy tissue.

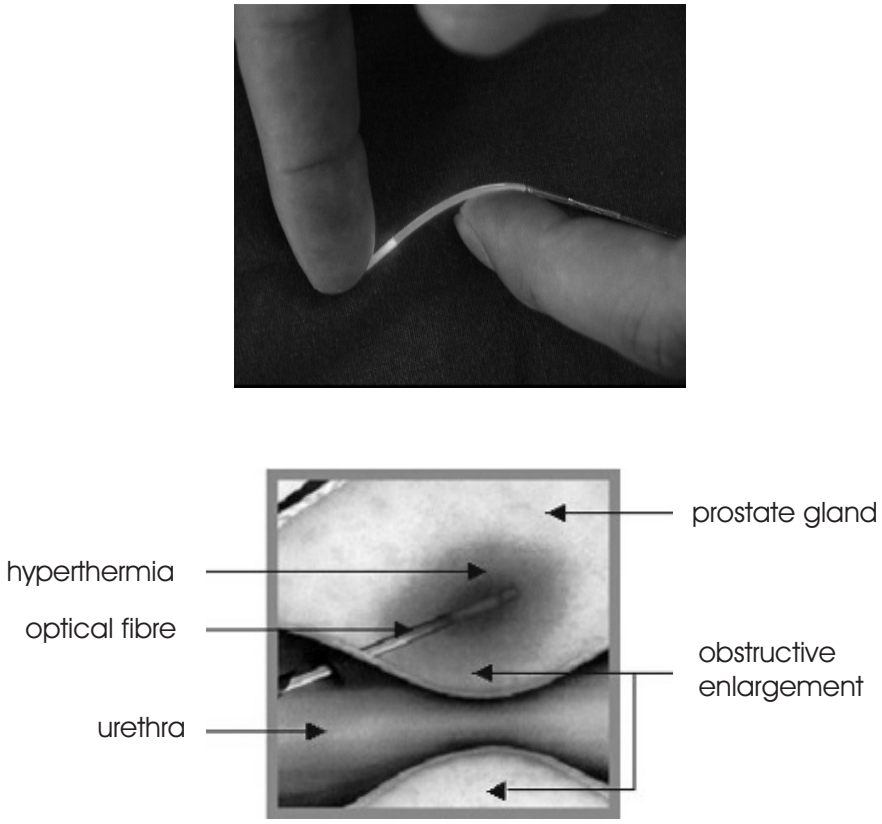


Figure 21: Optical fibre with a roughened end for use in laser-induced interstitial thermotherapy, and how it might be used to treat enlargement of the prostate.

5.5 Tissue Vaporization

The amount of heat deposited by a laser pulse incident on a piece of homogeneous tissue will decrease with distance into the tissue. The surface of the tissue will therefore heat most quickly. When the tissue reaches 100°C, the water will be vapourised. The remaining organic matter may then carbonise. (As soon as part of the tissue chars and turns black, the absorption coefficient there is much greater than in the surrounding tissue, and it will absorb the light strongly, thus accelerating the heating at this point.) The damage to the underlying tissue will depend on what temperature the tissue has reached, which in turn will depend on the length of the pulse and the thermal conductivity of, and perfusion within, the tissue. Often there are quite well defined regions of damage; Fig. 22 shows two scenarios. In the second, some of the water within the tissue has vapourised but cannot escape and forms vacuoles.

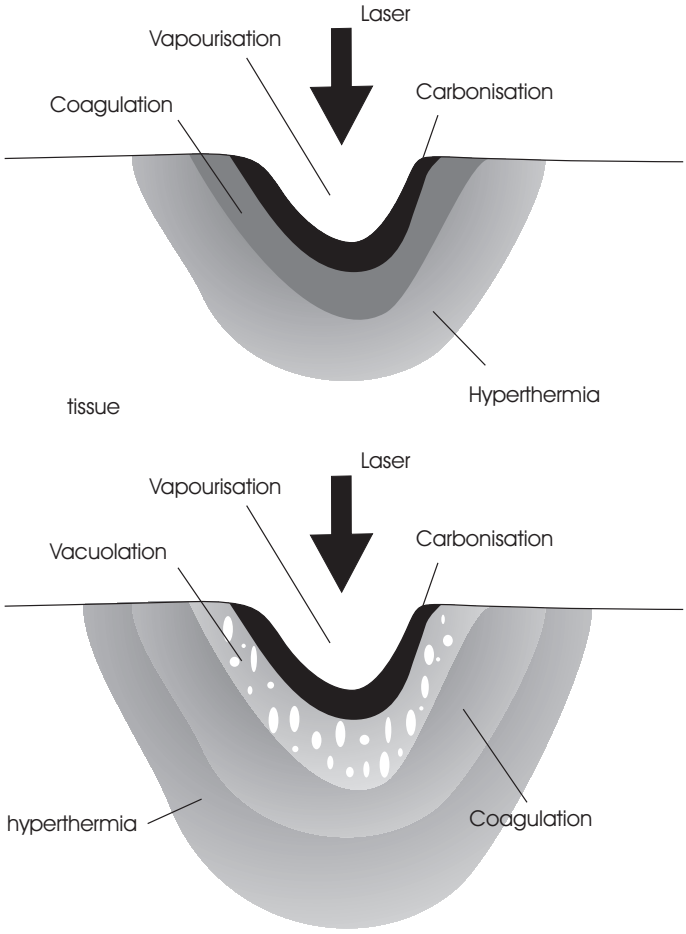


Figure 22: Spatial distribution of thermal effects following laser vaporization of tissue.

5.6 Example: Cutting with a CO_2 laser

A CO_2 laser, with a wavelength of 10.6 μm, may be used to cut tissue, by vapourising it. At this long wavelength water absorbs strongly ($\mu_a \approx 10^5$ m^{-1}) so the optical penetration depth is only $1/\mu_a \approx 10\mu$m into the tissue. The light energy is therefore all being deposited in a small volume of tissue, which rapidly heats up from 37°C to 100°C, absorbs the latent heat of vaporization, and is vapourised.

Consider a block of tissue being continuously illuminated. Once the top layer of tissue has been vapourised, the light will be illuminating the tissue (previously) beneath it, which will eventually be vapourised too. And so on. The exposed tissue about to be vapourised is said to have reached the *vaporization plane*. When a steady state has been reached, by which we mean the temperature distribution with respect to the vaporization plane is constant, heat diffusion does not need to be explicitly considered. In this steady state, the amount of tissue vapourised per second is constant, and we can estimate the speed with which the vaporization plane moves, ie. the rate at which the cut depth increases.

The energy per unit volume (energy density) required to raise the tissue by T K is

$$E_B = \rho C_p T. \tag{59}$$

The energy per unit volume required for the phase change is

$$E_L = \rho L \tag{60}$$

where L is the latent heat (or enthalpy) of vaporization ($L_{\text{water}} = 2.26 \times 10^6$ Jkg^{-1}). If the fluence rate, the energy per unit area per second being delivered to the tissue, is Φ_0 Wcm^{-2}, then the speed of the vaporization plane may be written as

$$\text{speed of cut (m/s)} = \frac{\text{energy incident on tissue per second (Jm}^{-2}\text{s}^{-1})}{\text{energy required to vapourise tissue (Jm}^{-3})}$$

or in symbols

$$v = \frac{\Phi_0}{\rho(C_p T + L)}. \tag{61}$$

This mechanism for ablating and cutting tissue is good because

- the cut depth can be finely controlled,
- and the blood vessels in the remaining tissue are coagulated with the heat, reducing bleeding.

(Do not confuse ablation of the tissue through *vaporization*, a photothermal effect that uses low energy photons, with *photoablation* described in the next section, on ophthalmology, which ablates tissue via direct breaking of chemical bonds with high energy photons.)

6 Lasers in Ophthalmology

The three mechanisms of laser-tissue interaction shown in Fig. 3 that we have yet to discuss are photoablation, plasma-induced ablation and photodisruption. As these mechanisms are used in ophthalmology more than perhaps any other discipline, this section, which covers laser applications in ophthalmology, will introduce them and some of their applications. The anatomy of the eye is shown in Fig. 23.

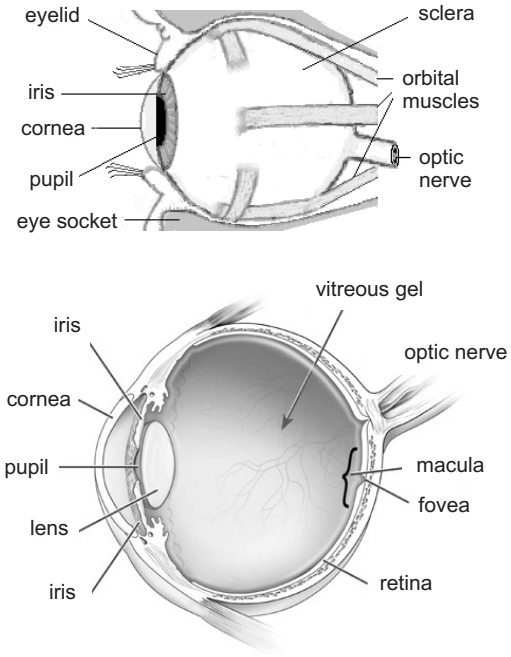


Figure 23: The anatomy of the eye.

6.1 Photothermal Treatments

Many diseases and medical problems of the eye can be treated using lasers in a thermal regime. Here are a few of the more common treatments:

• The back of the eye, the retina, contains a region called the macula which is the most important part for vision (it has the highest density of rods and cones). Damage to the macula leads to significant loss of vision. The retina contains many blood vessels. When too many blood vessels grow, *neovascularisation*, or a vein is occluded leading to a build up of blood, *central vein occlusion*, vision can be seriously impaired. By thermally photocoagulating the vessels, vision can be restored to some extent and the likelihood of recurrence can be minimised. When the whole of the retina - except the macula - is coagulated it is termed **panretinal photocoagulation**. This is a standard treatment for *diabetic retinopathy*, in which neovascularisation occurs.

• A *detached retina*, where the retina comes away from the back of the eye, can treated by 'gluing' it back on again by photocoagulating it at a number of spots (away from the macula).

• *Retinal tumours*, retinoblastomas, can be thermally necrosed.

• Glaucoma is caused by a build-up of pressure in the eye. *Closed-angle glaucoma* can be treated by making a hole in the iris, thus releasing the pressure. This procedure is called **laser iridotomy**.

6.2 Non-thermal Treatments

Thermal effects are not always desirable, particular when attempting to ablate or cut tissue very precisely without damaging the surrounding tissue. **Photoablation**, **plasma-induced ablation** and **photodisruption** are all used as non-thermal means of ablating or cutting tissue.

• **Corneal reshaping** to treat *myopia* or *hyperopia* (near or long-sightedness) is the commonest application of lasers to ophthalmology that uses a non-thermal mechanism. Three procedures are described below: radial keratectomy, photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK), all of which use photoablation as a mechanism to remove corneal tissue.

• Much research is being undertaken on the uses of laser-generated plasmas in ophthalmology and other fields. The difficulty in the past was that plasma generation with nanosecond or longer pulses was accompanied by photodisruptive, mechanical effects such as shock waves and cavitating bubbles that caused unwanted damage. Now that picosecond and femtosecond laser pulses can be generated routinely, it is possible to reduce and therefore control the extent of these photodisruptive effects. **Posterior lens capsulotomy**, in which an unwanted membrane across the back of the lens is broken, thus removing *secondary cataracts*, uses photodisruption as well as the advantage of plasma shielding.

6.3 Photoablation

Photoablation, or ablative photodecomposition, refers to a mechanism of laser ablation whereby the atoms bound together as molecules are dissociated through the direct breaking of the chemical bonds holding them together. If very short (ps) pulse durations are used, then there are no thermal effects associated with this process and it is therefore sometimes known as *cold ablation*. The fact that photoablation causes no thermal damage, and the very accurate etching that can be achieved, are the main advantages of this technique.

High energy UV photons from an excimer laser, eg. ArF laser, raise the bonding electrons into non-bonding orbitals. They can then either fluoresce (and fluorescence is often seen during laser ablation) or, at the very next molecular vibration following the electronic excitation, the two atoms previously bonded can separate, ie. dissociate. When the rate at which bonds are being broken, the rate of bond dissociation, is greater than the rate at which they reform, then photoablation can occur.

As Fig. 24 shows, the photon energy must be somewhat greater than the bond energy before dissociation will occur. The tables below gives some typical bond energies of some common bonds, and the photon energies for verious laser types. The most common bonds in collagen, the major constituent of the cornea, are C−C, C−N and C−O. (It is because the bond energy of N≡N is so high that air is so inert.)

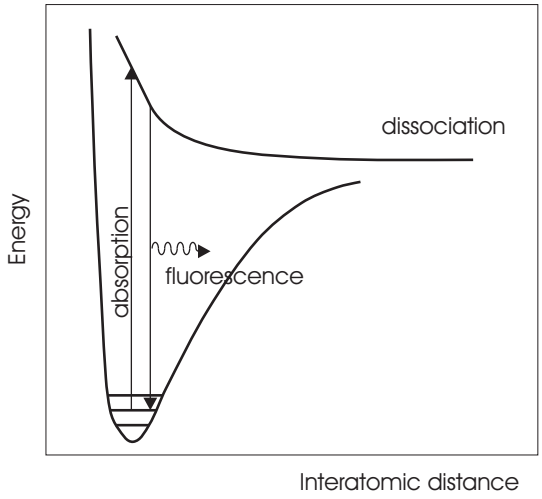


Figure 24: Photoablation relies on electronic excitation leading either to fluorescence or dissociation - bond breaking.

Bond	Energy (eV)	Energy (aJ)
N≡N	9.8	1.57
C=O	7.7	1.23
C=C	6.4	1.02
O=O	5.1	0.82
H−O	4.8	0.77
H−C	4.3	0.69
N=N	4.3	0.69
H−N	4.1	0.65
C−C	3.6	0.58
C−O	3.6	0.58
C−N	3.1	0.49
N−O	2.2	0.35
N−N	1.6	0.26

Laser	Wavelength (nm)	Photon Energy (eV)
ArF	193	6.4
KrF	248	5.0
XeCl	308	4.0
XeF	351	3.5
Argon ion	514	2.4
He-Ne	633	2.0
Ruby	694	1.8
Nd:YLF	1053	1.2
Nd:YAG	1064	1.2
Ho:YAG	2120	0.6
Er:YAG	2940	0.4
CO_2	10600	0.1

The bond energies and laser photon energies are put into perspective in Figs. 25 and 26. Clearly, to break the most common bonds will require high energy, UV, excimer lasers such as the ArF laser.

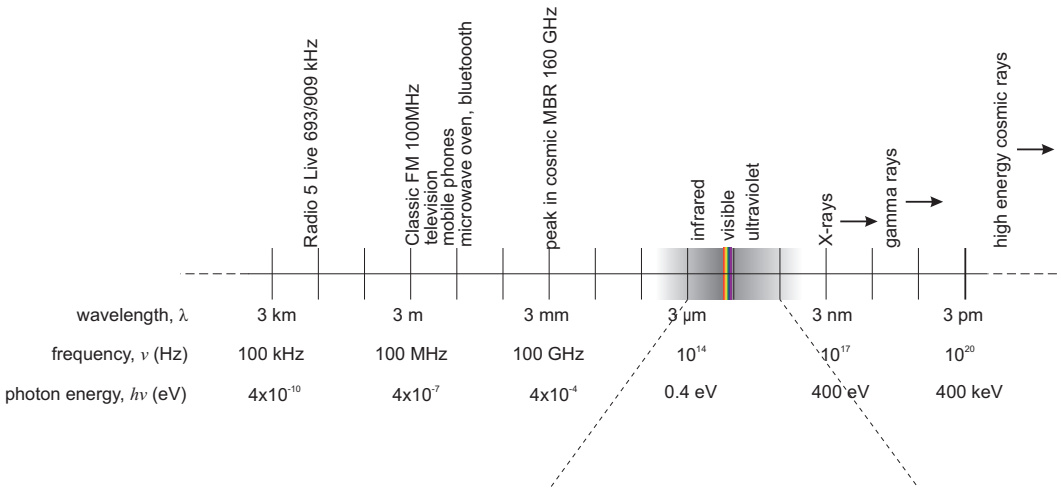


Figure 25: The electromagnetic spectrum. Frequency ν (nu) and wavelength λ (lambda) of light are related by the equation $c = \nu\lambda$ where ν is in Hz, λ in m and the speed of light $c \approx 3\times10^8$ m/s, and the energy E, in Joules, of a photon depends on its frequency, according to $E = h\nu$ where Planck's constant $h = 6.63 \times 10^{-34}$ Js. 1eV = 1.6×10^{-19} J.

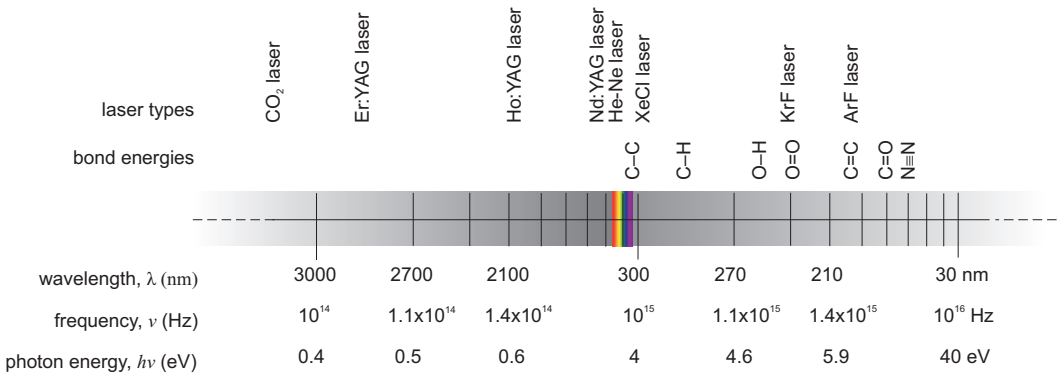


Figure 26: The infrared, visible and ultraviolet regions of the electromagnetic spectrum showing bond energies and the energy of photons from selected lasers.

6.3.1 Photoablation threshold

For tissue to be photoablated, the *rate of bond dissociation > rate of bond reformation*. Because the rate of bond dissociation is directly related to the number of photons incident on the tissue per second, which is proportional to the fluence rate Φ, there will be a threshold fluence rate below which photoablation will not occur.

Consider an illuminated absorber for which the fluence rate can be written as

$$\Phi(z) = \Phi_0 e^{-\mu_a z} \tag{62}$$

where Φ_0 is the fluence rate on the surface, μ_a is the absorption coefficient, and z is the depth from the surface. If the fluence rate threshold for photoablation is Φ_{ab}, then the depth to which material is ablated, z_{ab}, is

$$z_{ab} = \frac{1}{\mu_a} \ln(\Phi_0) - \frac{1}{\mu_a} \ln(\Phi_{ab}) \tag{63}$$

A graph of ablation depth z_{ab} against the log of the incident irradiance, $\ln(\Phi_0)$, is shown in Fig. 27. The onset of photoablation at the threshold fluence rate, Φ_{ab}, and the upper threshold beyond which further increases in irradiance do not lead to deeper ablation depths, are both marked. The latter is due to plasma formation, beyond which all incoming irradiation is absorbed by the plasma.

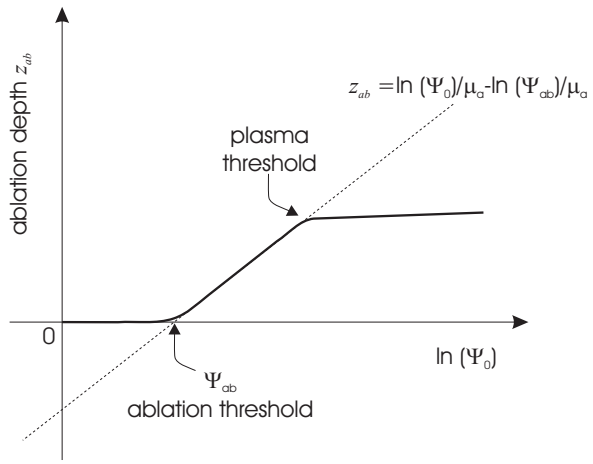
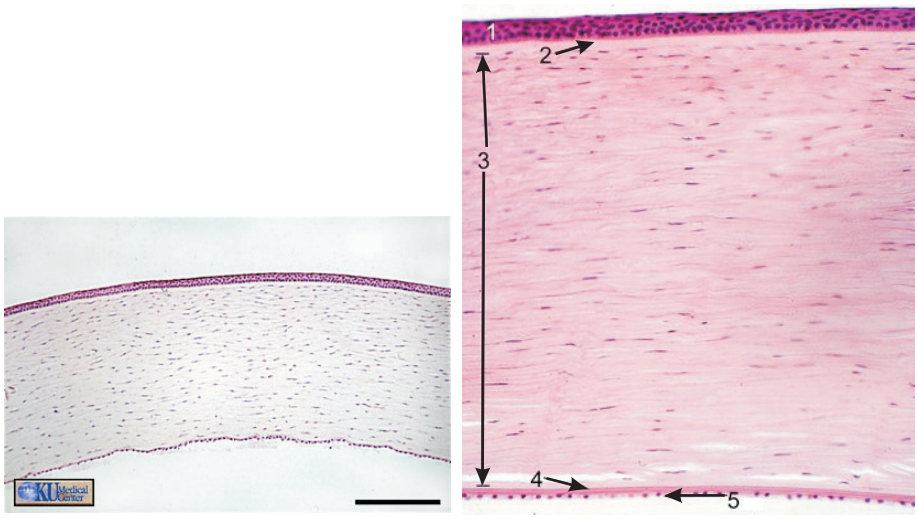


Figure 27: Ablation depth as a function of incident irradiance.

6.4 Refractive Corneal Surgery

The refractive power of the eye is approximately 2/3rds due to the cornea and 1/3rd to the lens. Refractive corneal surgery, in which the shape of the cornea is changed to affect the way it refracts light into the eye, in order to treat myopia, hyperopia and astigmatism, is perhaps the most common use of non-thermal photoablation in medicine. The anatomy of the cornea is shown below.



1. Epithelium, cells (50 μm)
2. Bowman's membrane, collagen (10 μm)
3. Stroma, collagen layers ($\approx$ 500 μm)
4. Descemet's membrane, collagen (5-10 μm)
5. Endothelium, single layer of cells (5 μm)

Before the use of lasers in ophthalmology, one treatment for myopia was a radial keratotomy, in which eight radial cuts were made on the cornea. Because of the change in the stresses on the eye caused by the cuts, the cornea flattened in the centre, thus correcting the refractive error causing myopia. When laser ablation became possible, the same procedure was repeated, but with the laser etching lines, rather than cutting them with a scalpel. This is called a **radial keratectomy**.

6.4.1 Photorefractive keratectomy (PRK) or LASEK

Rather than relying on the biomechanical properties of the eye to cause the cornea to flatten just the required amount, as radial keratectomy does, it is better simply to remove the required amount of corneal tissue and therefore directly change its refractive power. The first procedure to do this was photorefractive keratectomy (PRK), also called laser-assisted epithelial keratomileusis (LASEK or epi-LASEK), which is still commonly performed today. LASEK is different from LASIK discussed below. In PRK, the surgeon changes the refractive power of the cornea by

1. removing the epithelium mechanically (PRK) or softening it using an alcohol-based solution and rolling it out of the way (LASEK),
2. reshaping the superficial layer of the corneal stroma using excimer laser (photoablation),
3. and finally replacing the epithelium, or allowing the epithelium to grow back over 1-2 days.
4. A soft contact lens is often worn for a few days as protection.

Because the epithelium is replaced or grown back following the laser ablation, it acts to smooth the surface of the cornea, so there is no loss of visual acuity due to any slight roughness introduced by the laser ablation.

Problems with PRK include post-operative pain, the fact it cannot correct severe myopia or hyperopia, and regression of the refractive effect (which in practice means that too much corneal tissue is removed to account for the slight regression over the next few months, which means the patient has to cope with a number of different strengths of spectacles for a while).

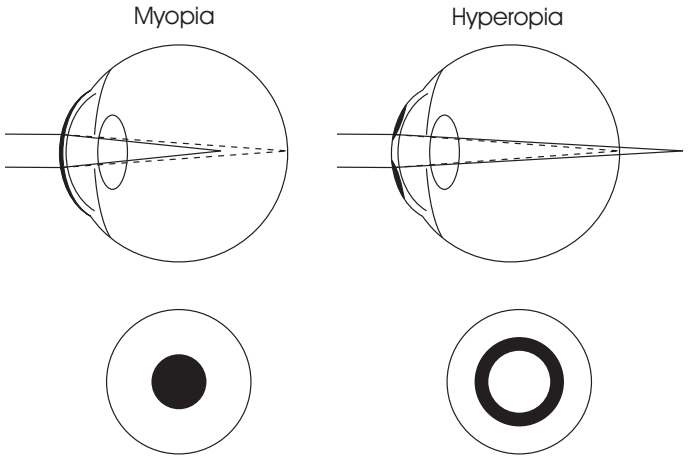


Figure 28: Photorefractive keratectomy procedures to correct myopia (near-sightedness) and hyperopia (long-sightedness).

6.4.2 Laser in situ keratomileusis (LASIK)

For large refractive corrections LASIK is better than PRK as it removes material from the interior of the stroma, not the surface, and so seems to suffer less from regression of the refractive effect. Also, because the epithelium does not have to regrow, there is less potential for scarring, and the treatment is complete with in a day.

The procedure is:

1. Remove ≈ 100 μm thick flap of corneal tissue using a microkeratome (a specially designed diamond knife),
2. reshape the corneal stroma using excimer laser (photoablation),
3. and replace the flap using tweezers. No glue is required as adhesion forces keep the flap in place until it has healed.

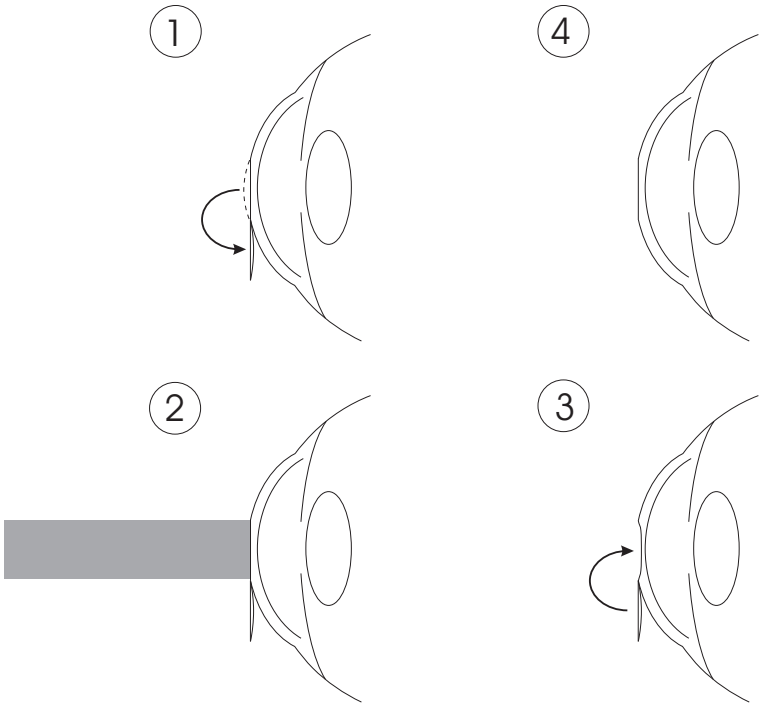


Figure 29: The stages in the LASIK procedure: flap removal, laser ablation, and flap replacement.

6.4.3 'Wavefront' or 'custom' LASIK

An improvement on LASIK, which customises the pattern of laser ablation to correct all the refractive errors of the eye, is called 'wavefront' or 'custom' LASIK. In this procedure is

1. low level visible light is reflected from a point on the retina,
2. an aberrometer is used to measure how much the reflected light is distorted by the lens and the cornea while travelling from the retina to the front of the eye,
3. the shape of the cornea that is necessary to 'undo' these aberrations is calculated,
4. the corneal stroma is shaped, just as in standard LASIK, but with a customised shape.

The idea is that if the light travelling from the retina to the front of the eye reaches it undistorted, the same will be true of light entering the eye. Wavefront LASIK therefore accounts for all refractive errors in the eye.

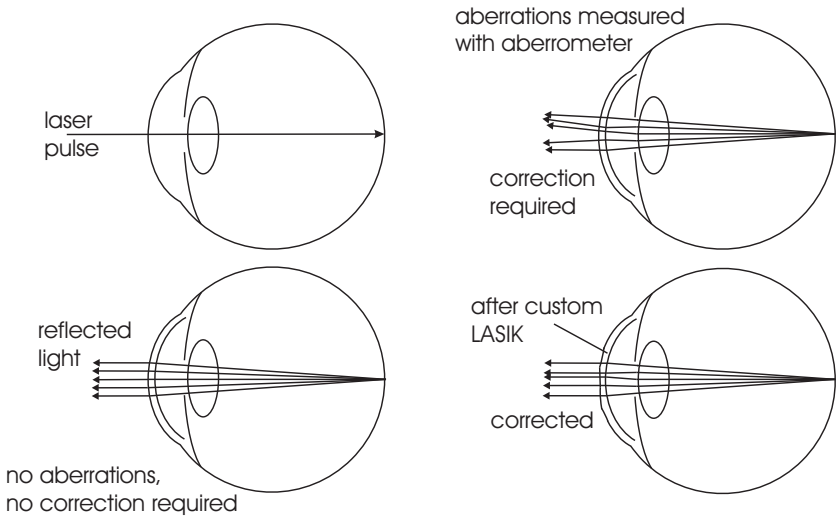


Figure 30: 'Wavefront' or 'custom' LASIK treatment uses an aberrometer to measure the abberations caused by the eye, so that they can be corrected by ablating the corneal stroma to just the right shape.

6.5 Plasma-Induced Ablation & Photodisruption

When the fluence rate reaches the plasma threshold, a plasma - a 'soup' of ions and free electrons - may form. In the days before picosecond and femtosecond laser pulses, the plasmas generated in this way were usually accompanied by photodisruptive effects, mechanical effects such as shockwave generation, cavitation bubble formation and bubble jetting. These additional effects were useful in some treatments (laser lithotripsy, the breaking up of kidney and bladder stones for instance) but for precision ablation they spread the damage over too large an area. Now that pico- and femtosecond lasers are available, plasmas can be generated while keeping the photodisruptive effects to a minimum.

The principal advantages of plasma-induced ablation, sometimes called plasma-mediated ablation or laser-induced breakdown, are that

- the thermal damage is minimal (as with photoablation),
- it is possible to ablate transparent tissue,
- when using fs and ps pulses the damage is well-confined,
- and it is good for cutting close to tissue that must not be damaged, as the high absorption of the plasma has a shielding effect.

Plasma formation requires free electrons. In strongly absorbing tissue, the linear absorption will lead to a slight increase in temperature and electrons will be realised through thermionic emission. In low absorbing (transparent) tissue which does not increase in temperature, multiphoton absorption is required to generate sufficient free electrons through multiphoton ionisation. This requires a very high fluence rate.

Optical Breakdown When there are free electrons in an electric field, optical breakdown can occur. Consider one free electron:

- the intense electric field due to the laser beam accelerates it,
- this highly energetic free electron now ionises another molecule by colliding with it, stripping it of an electron.
- Both these electrons are then accelerated into other molecules, etc, etc.
- Above a threshold fluence rate, at which the rate of ionisation > rate of recombination, a chain reaction leads to a plasma of free electrons and ionised molecules.

The threshold fluence rate depends on the pulse duration. Typical values for corneal tissue are

pulse duration	10 ps	100 ns
fluence rate	8×10^{11} Wcm^{-2}	7.3×10^{9} Wcm^{-2}
energy density	8 Jcm^{-2}	730 Jcm^{-2}

Note the high energy for ns pulses, which is the reason why the photodisruptive effects are greater for longer pulses.

6.5.1 Effects accompanying optical breakdown

- **Plasma** The generation of a plasma, spatially confined to the focal region of the laser, can be used to ablate tissue in a very controlled and precise way.
- **Plasma shielding** A plasma is highly absorbing because the free electrons can absorb any wavelength (their energy levels are not quantised). Because of this plasmas tend to absorb all incident light, and convert it into the kinetic energy of the electrons and ions, ie. heat. When performing a lens capsulotomy (breaking a membrane that has formed across the back of the lens following cataract surgery, a secondary cataract) plasma shielding prevents potentially damaging light from reaching the retina.
- **Shock wave** As the plasma grows rapidly, a pressure wave travels out into the surrounding tissue. The magnitude of the wave depends on the pressure and therefore the temperature in the plasma, which in turn depends on the energy density. For longer pulses, when more energy is deposited, these shock waves will be of greater amplitude.
- **Cavitation** A vapour bubble which forms around the plasma (for the plasma can become very hot) can grow to a critical size and then collapse violently, sending out another shock wave.
- **Jetting** If the cavitation bubble is close to a solid surface, it can form a jet as it collapses and 'fire' a jet at towards the solid surface at very high speed. (Such a jetting effect causes the damage seen on ships' propellers.)

These last three are *photodisruptive effects*, and are often undesirable, as they can cause damage well away from the focal region of the laser, where the plasma has formed. The photodisruptive effects are progresively less important as the pulse duration is made shorter. For picosecond pulse durations and fluence rates of $10^{11} - 10^{12}$ Wcm^{-2}, a plasma can be generated without the accompanying photodisruptive effects. For longer pulse durations and higher fluence rates, the photodisruptive effects are present. However, this mechanical energy can be used to advantage. For example, laser lithotripsy can be used to break up kidney stones.

7 Bibliography

This list is not intended to be a comprehensive survey of the literature of laser-tissue interactions. Because of the nature of the subject area, the relevant material is spread out over many different types of journals, from the medical and surgical journals with a more practical and clinical bias, to the physics and chemistry journals, which tend towards theory and experimental research. For up-to-date headlines and industry news try *http://medicalphysicsweb.org/*. UCL's research on photoacoustic imaging and other areas of biomedical optics can be found at *http://www.ucl.ac.uk/medphys/research/borl/*

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