Time Domain Diffuse Correlation Spectroscopy: models and experiments

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Abstract

In the past years, several spectroscopic techniques have been developed for the non-invasive measurement of tissue haemodynamic parameters in humans. The two mayor examples are near infrared spectroscopy (NIRS), that quantifies tissue composition (in particular haemoglobin concentration) and microstructure, and diffuse correlation spectroscopy (DCS), that quantifies blood flow (BF). Time domain near infrared spectroscopy (TD NIRS), using pulsed light sources, is capable of a depth-resolved measurement of tissue composition. Very recently a similar technique, time domain diffuse correlation spectroscopy (TD DCS), has been proposed for depth resolved and simultaneous evaluation of BF and tissue composition.

The aim of my thesis has been to validate the TD DCS technique and to extend its use from tissue-mimicking phantoms to in-vivo measurements on humans. This was done choosing an existing laser source, an actively mode locked Ti:Sapphire laser, that has the sufficient temporal coherence for the considered technique, and building the necessary TD DCS setup. I participated to the measurements and focused on the data analysis part. First, the technique was validated with the use of phantoms that mimic biological tissue. Then passing to measurements on humans, an in-vivo depth-resolved BF measurement, with a temporal resolution down to ~1 s, has been shown for the first time at my knowledge. Since this technique aims at absolute BF quantification, an accurate model for extracting the BF from the measurements is needed. For this reason, I focused on the study and the development of the correct theoretical model to be used for data analysis. This study was done with the help of numerical simulations.

This experimental and theoretical work opens the way to translation of the technique to practical use.
Sommario

Negli anni passati sono state sviluppate diverse tecniche spettroscopiche per la misura non invasiva dei parametri emodinamici del tessuto umano. I due esempi principali sono la Spettroscopia nel vicino Infra-Rosso (NIRS), che quantifica la composizione/struttura del tessuto e la Spettroscopia di Correlazione Diffusa (DCS), che quantifica il Flusso sanguigno (BF). NIRS nel Dominio del Tempo (TD NIRS), utilizzando sorgenti di luce impulsata, è in grado di misurare la composizione del tessuto con risoluzione in profondità (depth-resolved). Molto recentemente è stata proposta una tecnica simile, TD DCS, per la valutazione depth-resolved e simultanea delle concentrazioni dei costituenti del tessuto e del BF.

Lo scopo della mia tesi è stato di convalidare la tecnica TD DCS e di estenderne l'uso da fantocci simulanti il tessuto umano a misure in-vivo su esseri umani. Ciò è stato fatto con l'uso di una sorgente laser esistente, un laser Ti:Sapphire con mode-locking di tipo attivo, che consente di ottenere la sufficiente lunghezza di coerenza per la tecnica considerata. Il setup è stato adattato per le misure di TD DCS. Ho partecipato alle misurazioni e mi sono concentrato sulla parte di analisi dei dati. Innanzitutto, la tecnica è stata convalidata con l'uso di fantocci che imitano il tessuto biologico. Quindi, passando alle misurazioni sull'uomo è stata mostrata, per la prima volta a mia conoscenza, una misura del depth-resolved BF in-vivo, con una risoluzione temporale di ~ 1 s. Poiché questa tecnica mira alla quantificazione assoluta di BF, è necessario un modello accurato per estrarre il BF dalle misure. Per questo motivo, mi sono concentrato sullo studio e sullo sviluppo del modello teorico corretto da utilizzare per l'analisi dei dati. Questo studio è stato fatto con l'aiuto di simulazioni al calcolatore.

Questo lavoro sperimentale e teorico aprirà la strada al trasferimento della tecnica all'uso pratico.
1.1 - Diffuse Optics: Near Infrared Spectroscopy (NIRS) and Diffuse Correlation Spectroscopy (DCS)

Light was used to investigate materials already at the beginning of the last century (Cutler, 1929). Very early, optical techniques have been used to investigate human tissue, and to obtain different kind of information (Durduran T., 2010).

Light absorption is a decrease of light intensity due to the fact that, when light propagates in a medium, a part of its energy is taken by its constituents and thus converted from electromagnetic energy. Absorption depends strongly on the material and on the wavelength considered. The absorption of light is quantified by the absorption coefficient $\mu_a(\lambda)$, which is defined as the inverse of the ‘mean absorption length’, the typical distance that a photon travels before being absorbed. Since absorption of light is a consequence of the characteristic energy levels of the sample’s constituents, it depends on the spectrum and on the concentration of each constituent.

Light scattering is the variation in directionality of light when it impinges a particle or organelle within the medium. Scattering of light is quantified by the scattering coefficient $\mu_s(\lambda)$. Its inverse, the ‘mean scattering length’, is defined as the typical distance that a photon travels before being scattered (i.e. its direction is changed). Scattering of light is due to microscopic heterogeneities of dielectric constant, that occurs especially in biological materials (due to their peculiar structure). Thus, scattering provides information on the microscopic structure of the material. In a biological material light is highly scattered, and this kind of materials are called turbid media.

For describing the transport of light trough turbid materials, it is often used the reduced scattering coefficient $\mu'_s = (1 - g)\mu_s$, where $g = \langle \cos(\theta) \rangle$ is called anisotropy factor and is the average of the cosine of the scattering angle $\theta$ over all the possible photon paths within the medium (so called ‘ensemble average’). The inverse of $\mu'_s(\lambda)$ is called ‘random-walk step’ and is defined as the typical distance that a photon travels before its direction is randomized (i.e. it becomes completely uncorrelated with respect to the initial direction). The random-walk step is typically longer that the scattering length (since in a biological material typically one has $g \sim 0.7 – 0.9$), and it is also called ‘Photon transport mean free path’.

In biological materials, there exist an interesting range of wavelengths, between $650 – 950\,nm$, in which light absorption of the main tissue constituents (water, haemoglobin and lipid) is quite low (compared to the other wavelengths). Thus, in this range of wavelengths, called ‘physiological window’ (see Figure 1.1), light can penetrate deep into the tissue, up to several centimetres. Regarding to light scattering, photons can scatter either with static particles or with dynamic particles.
Figure 1-1: Human tissue light absorption. In the 600-1000 nm light absorption of oxygenated and deoxygenated haemoglobin (and also water, not shown) is low, and thus light can penetrate deep into the tissue. The spectral window between 700-900 nm is called ‘physiological window’. $\epsilon[c m^{-1}/M]$ is the extinction coefficient of each chromophore, i.e. its absorption coefficient per unitary concentration.

Near Infrared Spectroscopy (NIRS) studies scattering with static and dynamic (moving) particles, and light absorption. It is a spectroscopic technique that can be applied to several fields (food and agriculture, chemical industry, life sciences, medical and pharmaceutical, textiles) to test samples like liquids (in the food sector: oil, wine, and milk), powders (pharmaceutical tablets and pills, and wheat flour), and bulk objects (in the food sector: fruits and vegetables, meat, and cheese) (Torricelli A., 2014). It permits to characterize chemically the sample, finding which constituents are present and their concentration.

With the use of this technique, it is possible to measure important haemodynamic parameters such as the concentration of Oxygenated Haemoglobin ($O_2$Hb) and Deoxygenated Haemoglobin (HHb) in the tissue. This is possible since the absorption spectra of these two constituents are different one to the other (See Figure 1.1). From this, it is possible to compute important physiological quantities such as total haemoglobin concentration $tHb$ ($tHb = O_2$Hb + HHb) and blood oxygen saturation $SO_2$ ($SO_2 = O_2$Hb/tHb).

In NIRS, a weak light signal is injected into the tissue (Source) and the light that is re-emitted from the tissue is collected (Detector). The properties of the collected light carry important information on the tissue under study. In NIRS, typically, light is delivered and collected with the help of optical fibers. Regarding to the geometrical configuration, the experiment can be carried out either in transmittance or in reflectance geometry. Figure 1.2 shows the two possible cases.
In Medical applications, transmission mode is possible only in few cases such as in optical mammography (Antonio Pifferi, 2003) or, only in the case of infants, in the study of the brain (Jeremy C Hebden, 2002). Thanks to the high scattering of light, it is also possible to carry out the experiment in reflection mode. This permits the use of NIRS for many other Medical applications, such as the optical study of the adult brain.

In both geometries, there are three different kinds of light sources that can be used in a NIRS experiment. 1) CW (Continuous Wave) NIRS uses a steady state light source (i.e. intensity of light constant over time). In order to improve the sensitivity, light can be slowly modulated ($f \sim kHz$). In this case, it is necessary to use a phase-locked detection technique. A detector that can detect light attenuation, for instance a photodiode, is used. 2) FD (Frequency Domain) NIRS makes use of a frequency modulated light source ($f \sim 0.1 – 1 GHz$), and studies the phase shift and the amplitude change (with respect to the input signal) of the re-emitted light. 3) TD (Time Domain) NIRS uses a pulsed light source, with a duration of the order of $10 – 100 ps$, and the temporal profile of the re-emitted light (i.e. the pulse temporal broadening) is studied. As we will see later, the TD NIRS permits to separate different photon path lengths within the tissue, thus contains the largest amount of information per measurement pair. Figure 1.3 shows the three kinds of sources that can be used in NIRS.

![Figure 1.3: Three different types of light sources used in NIRS (Durduran T., 2010).](image)

Scattering with dynamic particles is studied by Dynamic Light Scattering (DLS). DLS studies the temporal intensity fluctuations of the light that is re-emitted from the tissue, for retrieving information on the motion of the scatterers (in particular their mean square displacement $< \Delta r^2 >$). The standard DLS technique have been used to characterize the motion of particles in optically thin, homogeneous samples. In the next paragraph I will describe more in detail an advancement of the DLS technique, Diffuse Correlation Spectroscopy (DCS).

In order to retrieve quantitative information from these experimental techniques (NIRS and DLS), is it necessary to exploit a theoretical model that describes how the light propagates within the tissue depending on the optical ($\mu_a, \mu_s'$) and geometrical (such as Source-Detector separation $\rho$) parameters. In fact, in biological materials, the transport of light is a complex interplay between absorption and scattering. The fundamental step was the understanding that, in this kind of materials, light propagation can be regarded as a diffusive process. Mathematically, this corresponds to say that light propagation obeys a diffusion equation, that in this specific case is called photon diffusion equation (PDE). Using this formalism, it is
possible to disentangle absorption from scattering. I will discuss the basic theory of photon propagation in Chapter 2.

As said before, the study of the fluctuations of light emerging from a medium permit to retrieve information on the motion of scatterers. In the case of tissue, the main contribution of this dynamic scattering is given by the Red Blood Cells. In DLS, the measured quantity is the intensity autocorrelation function of the re-emitted light, or its power spectrum. The motion of RBCs gives us a measure of the blood flow. As we will see in section 1.2, the first experimental techniques were constrained to single-scattering measurements, thus enabling the quantification of blood flow only for very superficial tissue (< 1 mm), as for instance in Laser Doppler Flowmetry (LDF).

The development of a theory for the multiple-scattering case permitted the birth of the Diffuse Correlation Spectroscopy (DCS) technique. DCS consists of the measurement of the intensity autocorrelation function of the diffused light and permits the characterization of deep tissue, since it is not anymore limited by the single-scattering hypothesis. The electric field autocorrelation obeys to an equation called Correlation Diffusion Equation (CDE), and this permits the quantitative evaluation of the BF from correlation measures. In fact, as we will discuss in chapter 2, the decay rate of the autocorrelation function depends strongly on the BF, see Figure 1.4.

![Figure 1-4: Example of a typical autocorrelation function, plotted using the solution of the CDE (with \( \rho = 1 \text{ cm, } \mu_a = 0.1 \text{ cm}^{-1}, \mu_s' = 10 \text{ cm}^{-1} \)). The two curves correspond to a low and high flow (\( D_B = 1 \times 10^{-9} \text{ and } 1 \times 10^{-8} \text{ cm}^2/\text{s}, \text{respectively} \)). Note how a change in BF correspond to a change in the decay rate of the autocorrelation function.](image)

One of the main applications of DCS is the measurement of Cerebral Blood Flow (CBF) in fact, in the physiological window, light penetration is enough to go through the skull and reach the cortex.

In section 1.2, I will make a comparison between the Diffuse Optics techniques (NIRS and DCS mainly) and other techniques for the evaluation of haemodynamic quantities, and discuss the medical applications of Diffuse Optics.

It is important to notice that, as we will discuss in section 1.3, the main drawback of DCS is that is not able to get depth resolution, at least with a single source-detector separation measurement, since it uses a Continuous Wave source (CW) and thus is not able to resolve
the different path lengths that the photons undergo in the tissue, and also for this reason it is affected by Partial Volume Artefacts. On the other hand, NIRS (and in particular TD-NIRS), using a pulsed light source is able to resolve the different path lengths of the photons. The main advantage of being able to resolve the path length in a TD NIRS experiments is that we can recover depth-resolved information regarding the absorption and scattering coefficient distributions. A similar possibility in DCS (but in this case for the Blood Flow) would be very attractive and will be discussed in particular in Section 1.3.

1.2 - Diffuse Optics in Medicine

Diffuse Optics techniques have a wide range of Medical Applications. Being able to determine haemodynamic quantities such as Oxy - Deoxy haemoglobin concentration and Blood Flow, it is able to both monitor and study several parts of the human body. In this chapter, I will first compare Diffuse Optics with existing techniques for monitoring haemodynamic parameters, and then I will discuss some specific applications of Diffuse Optics.

1.2.1 – Existing techniques for measuring the Blood Flow

Correct blood delivery is critical for every organ of the body, and in particular for the brain (Durduran T., 2010). Cerebral well-being is dependent on the correct supply of oxygen, and on the removal of the by-products of oxygen consumption (such as carbon dioxide). In a simplified picture, the arteries supply oxygenated blood, the exchange of oxygen with the tissue takes place in the capillaries, while the veins remove the deoxygenated blood. The whole process is controlled by the body with a mechanism called cerebral auto-regulation (CAR), that permits to keep the Blood Perfusion constant, even if other parameters such as the Local Pressure are changing (Meeri N. Kim, 2014). This is why it is so important to have a technique that is able to monitor the haemodynamic parameters, in particular at the microvascular level. In fact, the exchange of oxygen with the tissue takes place in the capillaries, and the brain needs to be supplied with oxygen in a continuous and controlled way to stay healthy.

In addition to that, the study of haemodynamic response to, for instance, functional activation or exercise, is important from a medical viewpoint. In the case of the brain, one very interesting question is how the neuronal activity is coupled to haemodynamic response. This coupling is called Activation Flow Coupling (AFC) and still needs to be studied in detail. Microvascular Blood Flow is difficult to monitor, in particular at the bedside. In this paragraph, I am going to discuss the main techniques for monitoring of the Blood Flow.

1) Laser Doppler Flowmetry (LDF): this technique is based on the spectral broadening of the light’s spectrum when it passes through a turbid medium. This technique is able to measure the microvascular BF, but it is constrained to single-scattering measurements. For this reason, its penetration depth is only ~ 1 mm, and thus it is necessary to remove the skull for being able to measure the cortex. Laser Speckle Flowmetry is a similar technique that uses a CCD camera, but for now it has been applied only to small animals.

2) Doppler Ultrasound (DU): it exploits the same physical principle of LDF (spectrum broadening due to scattering with moving particles), but it uses an ultrasound beam instead of light. It is able to measure blood velocity (and not flow), and it is limited to large vessels. Furthermore, the signal depends on the insonation angle, thus additional care is needed during the measure for keeping it constant. In the case of the brain, Transcranial Doppler
Ultrasound (TDU) is able to monitor the CBF at the bedside, but since it measures velocity and not flow it has limited applications when the diameter of the vessel changes (for instance in stroke).

3) Arterial Spin Labelling MRI (ASL-MRI): It is a Magnetic Resonance technique that is sensible to flow, and it is possible to use for the brain. The main advantage of this imaging technique is that is Region of Interest (ROI) is very large: it is possible to have images of the whole brain. The main drawback of this technique is that its time of acquisition is long (~30 min), and being the machine big and expensive it is difficult to have continuous measurements at the bedside.

4) Positron Emission Tomography (PET) is a technique able to measure directly blood flow and metabolic rate, and has similar characteristics to MRI. The drawback of PET is the use of radioactive materials and external contrast agents.

5) Xenon Computed Tomography (Xe CT) is another ‘full-head’ technique (i.e. that images the whole brain). It uses a Xenon gas as the contrast agent. As MRI and PET, the instrument (and the contrast agents) are quite expensive. This limits the applicability of this technique to bedside measurements.

As it is possible to understand from the discussion, an ‘ideal’ technique for BF measure does not exist, and Diffuse Optics techniques could be a good solution for a fast and non-invasive diagnostic method to be used in the clinic. In the next section, I am going to discuss the advantages of Diffuse Optics (in particular DCS) for the measure of BF and some of its limitations.

1.2.2 – Measuring microvascular Blood Flow with DCS

As pointed out before, Diffuse Optics techniques can be used to measure important haemodynamic quantities. In particular, DCS measures Blood Flow, and has been validated against several existing techniques.

DCS has been validated against Xenon CT. The comparison has been carried out, for example, in the intensive care unit (ICU) during intervention on injured-brain adult subjects (Kim, 2010). It has been shown that the relative variation of the blood flow index from the two techniques have a good correlation.

DCS has been validated also against TDU. As an example, in a study on the brain of pre-term infants (Buckley, 2009), it has been shown that the Blood Flow measured with DCS is well correlated with the peak systolic velocity measured with TDU. In the TDU measure, typically the Middle Cerebral Artery is insonated. Thus, this study shows an interesting relation between micro and macro-vascular CBF.

Another validation has been carried out with ASL-MRI. In a study (Yu G., 2007), the correlation between ASL signal ad DCS signal in muscular tissue has been evaluated. In the study, the flow during a cuff occlusion has been measured using both the techniques. Cuff occlusion is a protocol to study the response of muscular tissue: during the occlusion, a short ischaemia is forced to the muscle, and a hyperaemic response follows immediately after the release of the occlusion itself. The flow values measured with the two techniques showed similar temporal trends during the experiment. In particular, after the release of the occlusion, the flow values at the hyperaemic peak showed a strong correlation.
These examples show that DCS is a reliable method for measuring Blood Flow. More in general, Diffuse Optics has some advantages with respect to the existing techniques for the measure of hemodynamic parameters (Durduran T., 2010). First, Diffuse Optics methods are non-invasive and have no risks for the patients. Second, Diffuse Optics methods can be used in real-time. This feature is particularly attractive in some environments like Intensive Care Unit (ICU) where a fast measurement is necessary to assess the status of the patient and decide the correct protocol. Third, it is possible to use continuously these techniques for a long period of time (up to hours/days). Finally, yet importantly, the technique is applicable at the bedside and it has a moderately low cost. Another peculiarity of Diffuse Optics methods is the high temporal resolution, that can go down to ~0.1 s.

Also, some limitations of these techniques exist: the spatial resolution is lower than other imaging techniques as MRI, and being based on light, it is not able to measure the deep brain (since the maximum penetration is of the order of 3 cm). Additionally, it is still necessary to validate these methods in large clinical trials to gain general acceptance.

Diffuse Optics is able to detect focal changes in haemodynamic response in the brain. This possibility has been explored for different kinds of functional activation such as vision, movement and verbal fluency activations. This is important because neuronal activation is coupled to vascular activation through a phenomenon called Activation Flow Coupling (AFC). To understand in a clear way the neurovascular coupling, it is necessary to know, possibly in an independent way, several haemodynamic quantities like the oxygen saturation and the Blood Flow. The combination of these two pieces of information (i.e. the combination of NIRS and DCS) permits to compute in an accurate way the cerebral metabolic rate of oxygen consumption $\text{CMRO}_2$ (Mesquita R., 2011). Using a combination of DCS and NIRS it is possible to compute the $\text{CMRO}_2$ in a more accurate way with respect to the two techniques used separately. In any case, it is only possible to determine the relative variation of the metabolic index, not its absolute value. Metabolic rate has been successfully measured before in small animals, and after in humans. The metabolic rate measurement using Diffuse Optics has been validated against MRI and PET, and showed good correlation. The critic advancement in the measurement of $\text{CMRO}_2$ with Diffuse Optics is the addition of DCS, because the metabolic rate is proportional to CBF. Without DCS it is necessary to make additional hypothesis to obtain a quantitative value.

The use of several probes permits also to obtain tomographic (3D) or topographic (2D) images of the hemodynamic parameters. In particular, is possible to obtain maps of the absorption and scattering coefficient in the tissue, using a technique called Diffuse Optical Tomography (DOT). This technique has been successfully used for localizing focal changes in various medical scenarios, for instance in optical mammography and functional brain activation. The DCS counterpart of DOT is called Diffuse Correlation Tomography (DCT). With the use of several probes on the surface of the tissue, DCT permits to obtain 3D images of the Blood Flow. This technique tries to overcome the main limitation of the “standard” DCS technique, the lack of depth resolution. The main disadvantage of this technique is the need to use several source-detector pairs.
Cerebral autoregulation

In the last few years, Diffuse Optical methods has been used for investigating cerebral auto-regulation. Cerebral auto-regulation is the ability of the brain to keep its Blood Flow in an optimum range, despite external disturbances. In addition to that, several studies have been done for cancer therapy monitoring, and more in general for improving patient care. As already pointed out before, the clinical tools for monitoring the brain can be very invasive, for instance Intracranial Pressure (ICP) monitoring typically needs to access directly to the surface of the brain, drilling a hole in the skull. On the other hand, MRI and PET are not readily usable at the bedside. One clinical application example is the monitoring of Acute Ischaemic Stroke (AIS). AIS patients have an impaired cerebral auto-regulation. For this reason, CBF is not stable anymore, and depends on cerebral perfusion pressure.

One method for checking the cerebral auto-regulation of these patients is the Height of Bed (HOB) challenge. HOB challenge consists of changing the angle of inclination of the bed in which the patient is resisting, while measuring the CBF. Analysing how the CBF changes as a function of the bed’s angle permits to obtain useful information about the auto-regulation impairment of the patient.

One last example of clinical application of Diffuse Optics is the monitoring of haemodynamic responses of tumours during treatment, in particular at the beginning of the therapy itself. Measuring the blood flow permits to estimate the drug delivery efficiency. In addition, the measure of tumour oxygenation permits to obtain useful information about the status of the illness.

1.3 – Depth resolution in DCS: is it possible?

In the previous section, I have discussed the medical utility of DCS for measuring the Blood Flow. It is important to notice that the “standard” DCS consists in the use of a steady-state light source (CW). In the case of NIRS, a CW source is not able to resolve the different photon path lengths, and thus it does not have resolution in depth. This concept holds true also in the case of DCS. In the next chapter, I am going to discuss some experimental advancements of DCS that try to overcome this limitation. On the other hand, as I will discuss in this section, TD NIRS is able to resolve different path-lengths, and the combination of DCS with TD NIRS could be very promising for obtaining a depth-resolved Blood Flow. Now I am going to introduce the physical principles of TD NIRS, and then how it could be possible to combine DCS with TD NIRS.

TD NIRS relies on the measurement of the photon Distribution of Time of Flights (DTOF). A short light pulse is injected into a turbid medium and then measured, after propagation into the medium, at a certain fixed distance $\rho$ from the source (typically $10 - 40 \, mm$) (Torricelli A., 2014). The pulse, due to the propagation, is delayed, broadened and attenuated, see Figure 1.5 (a). The region of most probable photon paths, sometimes called ‘banana shape’ is showed. The delay effect is present because light propagates at a finite speed

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1 The (photon) Time Of Flight (TOF) is defined as the temporal delay at which a photon is detected with respect to the time of its injection into the tissue.
into the tissue. The broadening effect is due to the multiple scattering that the photons undergoes in all the possible photon paths. The attenuation is due both to absorption (because it decreases the probability of detecting the photons) and scattering (because the photon trajectories are deflected in directions different from the initial one). However, it is worth noting that if there were no scattering at all, we would get no signal in reflection geometry, thus scattering is somehow useful for us.

Increasing the Source-Detector (SD) separation decreases the number of detected photons and increases the temporal broadening and the delay of the re-emitted pulse. Similar dependences are observed when the scattering is increased. Absorption, on the other hand, affects the number of photons detected and the slope of the tail, but it does not change significantly the position of the peak. Figure 1.5 (b,c,d) shows a summary of the dependence of the DTOF on the geometrical and optical parameters. The curves showed are calculated assuming a homogenous semi-infinite medium. In reality, a biological system is never homogeneous. In the case of the head, for instance, the system is composed of several layers (scalp, skull, and cortex) with different thickness and optical properties. The effect of these heterogeneities needs to be described by suitable theoretical models.

The photons that are re-emitted by the turbid medium probe a region that have roughly a ‘banana shape’. Typically, with CW sources, it is possible to increase the depth sensitivity by increasing the SD separation. In TD NIRS, it is possible to improve the depth sensitivity by exploiting the time resolution in photon TOF. In fact, the probability that photons have reached a certain depth $z$ after propagation $p(z,t)$ depends on the selected time (and it is possible to show that it does not depend on the SD separation $\rho$). In particular, this probability increases over time, thus selecting photons with sufficiently high TOF is it possible to obtain a larger sensitivity from deeper regions (Martelli F., 2016). Thus, in a TD NIRS experiment, the temporal information permits to resolve different path lengths. Longer path lengths statistically correspond to photon that propagated deeper into the tissue.

As it is possible to understand from the previous discussion, the combination of TD NIRS and DCS can be a very promising solution to the lack of depth resolution of “standard” DCS. In this paragraph, I am going to discuss the concept of the experimental technique that arises from this combination, called Time Domain DCS (TD DCS), which is the main subject of my Thesis work. In Chapter 2 I will discuss in more detail both this technique and other previous techniques that have been used to address the problem.

In TD DCS, a train of light pulses is injected into the tissue, and then collected at the detector side. With the use of temporal gating, only a certain time interval of the DTOF is selected, see Figure 1.6. This time interval will correspond to a certain path length interval into the tissue. After this temporal gating, the intensity autocorrelation function of the re-emitted light is measured (Sutin K., 2016). With respect to standard DCS, we could call this function gated autocorrelation. As it is possible to understand from the previous discussion, since the different gated autocorrelations correspond to different time intervals, and thus to different path lengths, these functions will carry information about different depths into the tissue. In particular, by selecting the earlier part (early photons) of the DTOF, we will get an autocorrelation curve that will be more sensitive to the superficial flow. On the other hand, by selecting the later part (late photons) of the DTOF the autocorrelation function will be more sensitive to deeper flow. Of course, it also possible to compute the autocorrelation function of the whole DTOF, and this function will be sensitive to both superficial and deeper flow (in general in different proportions).
One of the most difficult requirements to fulfil in order to build a TD DCS setup is the coherence of the light source. In fact, DCS measurement is based on the speckle pattern fluctuations of the re-emitted light from the tissue. A laser speckle is the interference pattern of laser light that is created when the light is diffused through a scattering medium (Svelto, 2010). Its physical origin is due to the constructive and destructive interference of the light rays when they arrive to the surface of the medium. Since some of the scatterers are moving, the speckle pattern will fluctuate over time, and its statistical properties will carry information on the scatterers’ motion. In fact, light scattering is a deterministic process (even if it is more convenient to describe it mathematically as a stochastic process), and a certain displacement of a scattering centre will give rise to a specific change in the light pattern that is observed at the surface of the medium. This is the physical principle over which DCS is built. In “standard” DCS experiments, since we do not apply temporal gating, all the path lengths can contribute to the interference pattern and thus to the measured intensity autocorrelation function. In this case, in order to have interference on the detector side, the coherence length of the laser source has to be longer than the maximum difference of the path lengths that the photon undergo within the tissue (T. Bellini, 1991). In fact, path lengths that differ one from the other for more than a coherence length will interfere only partially and thus give a negligible contribution to the measured autocorrelation function. Typically, a steady-state light source can have a very long coherence length (of the order of meters), thus this coherence requirement is not very difficult to fulfil with commercial CW lasers. On the other hand, in TD DCS it is necessary to use a pulsed laser source. Due to the short duration of the signal, this kind of source intrinsically has a shorter coherence length with respect to a steady-state source. To obtain a pulsed laser source with the sufficient coherence length is not trivial,
and for this reason it has been belief for some time that TD DCS was not experimentally possible.

In reality, as I will show in the next Chapters, is it possible to build a high coherence pulsed laser source that permits to fulfil the coherence requirement (M. Pagliazzi, 2017). The choice of the pulse duration is critical, because shorter pulses make easier to determine the tissue optical properties with TD NIRS analysis, but diminish the source coherence degrading the quality of the measured autocorrelation functions.

An important point is the exploitation of the temporal gating, which can help to diminish the coherence requirement (Sutin K., 2016). In fact, when we build the gated autocorrelation, it is only necessary that the path lengths within the gate interfere, and not the path lengths outside. This because only the path lengths that are not rejected by the gate contribute to the measured intensity fluctuation and thus to the resulting autocorrelation function. Thus, in this case the coherence length needs to be greater than the difference between the minimum and maximum path lengths of the gate. Consequently, making the gates narrower will decrease the spread in path lengths, and this can help to overcome the coherence requirement.
Chapter 2 – State of the Art

In This Chapter I will discuss the State of the Art of Diffuse Optical methods for measuring haemodynamic parameters. In particular, In Section 2.1 I will discuss the various advancements of the DCS techniques, which try to overcome the main drawback of DCS, when used with CW light sources: the lack of depth resolution, if used with a single SD separation. In Section 2.2 I will discuss the present advancements of TD DCS, a technique that have been introduced already in Section 1.3. In Section 2.3 I will discuss an advancement of TD NIRS technique, the use of fast-gated detectors, which potentially could also be applied to TD DCS.

2.1 – Techniques for separating superficial from deep blood flow in DCS

As I already discussed in the previous Chapter, standard DCS uses a steady-state laser source. For this reason, it has not the possibility to resolve different path lengths within the medium. The main consequence of this is the lack of depth resolution. In the literature, several methods have been developed to try to overcome this limitation. In this Section I will discuss the four main methods that have been used.

2.1.1 – Partial Volume Effects correction in DCS

In Diffuse Optical methods (both NIRS and DCS), when a steady-state light source is used, light propagates and probes different kind of tissues, without discriminating between tissue types, for instance bone and brain, or skin and muscle (Turgut Durduran, 2004). This can bring to a phenomenon called Partial Volume Effect (PVE), in which the values of the hemodynamic parameters are wrongly estimated. In fact, without pulsed light sources, light propagates at different depths within the tissue, and at the detector side all this path lengths are mixed. Thus, it is impossible to decouple the deep and superficial contributions from the measured signal. In addition to that, also tissue inhomogeneities can affect the correct estimation of hemodynamic parameters, in the case of focal changes (for instance functional activation). Comparing Diffuse Optics and MRI signals, it has been shown that PVE can bring to significant reduction (>2 times) of the estimated haemodynamic parameters.

In the case of DCS measurements, PVE effects can distort our estimation of the BF. The PVE are basically due to the fact that the light that probes deeper layers needs in any case to travel also through the superficial layer. One case in which this can bring to significant errors in the BF is the head. In fact, the head is a strongly heterogeneous system, composed of low BF regions (scalp and skull) and high BF region (brain). Typically for the data analysis a homogeneous model is used for the estimation of the BF values, disregarding the layered structure of the considered system. Both Monte Carlo simulations and in-vitro experiments showed that this can bring to an underestimation of the CBF (i.e. flow of the brain) by approximately 5 times. For compensating for PVE, first a correction factor is computed on the basis of simulations or experiments, then this factor is used to correct the BF value retrieved from the actual experiment. This method has the constrain that, for an accurate correction, the system for estimating the correction factor and the system used in the actual experiments need to have similar properties.
Another possible method for reducing the PVE is the use of advanced data fitting of the autocorrelation function, for instance the use of a Penalty function (Pau Buch, 2013). The use of this method consists in choosing in the autocorrelation functions only the parts that are more sensitive to deeper flow changes. In fact, as we will see later, smaller correlation times $\tau$ corresponds to photons that have travelled longer paths, and thus have a larger probability to have probed deeper regions. In a similar way, it is also possible to use the method for estimating the superficial flow. We will see in Chapter 5 that this concept is somehow useful also in TD DCS to enhance the deep resolution of the technique. The main limitation of this technique is that, even if different parts of the autocorrelation carry information about different path, we are still using a steady-state light source, so it is impossible to completely decouple the path lengths simply by selection of the correlation time. Thus, the estimation of the BF with this method does not bring a perfect estimation of the BF values for deep and superficial layers.

2.1.2 – Diffuse Correlation Tomography

Another way for overcoming the depth-resolution limitation of the standard DCS is called Diffuse Correlation Tomography (DCT). DCT is the tomographic version of DCS, i.e. the aim of the technique is to obtain a 3D image of the Blood Flow. This is done with the help of several sources and detectors placed on the surface of the system to be imaged. The technique is similar to Diffuse Optical Tomography (see Chapter 1), the difference is that it creates images of the Blood Flow and not of the tissue optical properties. With respect to DOT, Blood Flow imaging is more difficult to obtain due to sensitivity to noise and to data selection (Chao Zhou, 2006).

DCT uses a pattern of sources and detectors placed on the surface, and one autocorrelation curve is measured for many source-detection pairs. Specifically, the variations in Blood Flow are estimated by comparing the “perturbed” and “unperturbed” autocorrelation functions for each source-detector pair. The theory of DCT is based on the Correlation Diffusion Equation (CDE), which will be introduced in Chapter 3. One important point is that, when the tissue flow properties are heterogeneous, any point in the medium gives a contribution to the measured autocorrelation. The coupling between space-dependent flow changes and the measured autocorrelations is given by a suitable sensitivity matrix. This matrix, if inverted, permits to compute the value of the flow in each voxel of the considered domain. Since the inversion of this matrix is ill posed, the choice of suitable regularization strategies is critical.

The main limitation of DCT is that it needs to use several source detector pairs to create an image of the BF. In some applications, this cannot be possible, due for instance to space constrains. Thus, a method that does not exploit different sources and detectors is preferable for building more compact medical devices for the measurement of the Blood Flow. Furthermore, for now DCT has been applied mainly to small animals and not to human studies.
2.1.3 – Extra-cerebral contribution estimated applying pressure to the probe

In a DCS experiment, the measured blood flow is a combination of superficial and deep flow. Quite recently, a study characterized the effect of superficial flow on DCS signal, in particular in the case of the brain (i.e. extra-cerebral BF), and the effect of varying the pressure applied to the probe (Rickson C. Mesquita, 2013).

In order to understand extra-cerebral contributions to the measured BF, a model that describes how deep and superficial flow impact on the total flow signal needs to be used. The fact that superficial blood flow is sensitive to probe pressure permits to extract the extra-cerebral part of the total signal, by taking measurement at different probe pressures. In particular, the use of high pressures is expected to reduce the superficial flow, and thus can permit more accurate estimation of relative changes in CBF.

The experimental protocol consists in two different measurements: one with pressure applied to the probe and one without pressure. Several source detection (SD) separations are present in the used probe. This permits to have two degrees of freedom (applied pressure and SD separation), thus facilitating the decoupling of extra-cerebral and cerebral BF. The model developed in this study expresses the total flow signal as a sum of extra-cerebral and cerebral flows. The cerebral contribution is multiplied by an “efficiency” factor, which considers that the deep layer contributes in a different way with respect to the superficial layer, depending on the particular SD separation used. In the model it is assumed that the extra-cerebral contribution does not depend on the SD separation, but depends on the probe pressure. On the other hand, it is assumed that the cerebral contribution does not depend on the applied pressure, but depends on the SD separation only through the multiplicative efficiency factor.

The use of this model permits to study better the effect of extra-cerebral contribution, for example by taking measurements at small source detector separation and for different applied pressures. This information permits then to have an estimation of the extra-cerebral contribution at larger SD separations. On the other hand, measurements at different SD separation permit to study the CBF contribution to the total signal. This method can also be applied to study dynamic changes. In particular, for measurements at high pressure applied (if extra-cerebral contributions are negligible), the relative variation in the total signal can be shown to depend only on relative variations in the CBF signal. This permits direct estimation of dynamic changes in cerebral flow.

As it is possible to understand, the main drawback of this method is that it needs several measurements (at different SD separations and applied pressures) to estimate the CBF. This can be limiting in a clinical setting due to low amount of time at disposal or to probe dimension constrains. In addition to that, data analysis is dependent on several assumptions that can be not valid in all the tissue types and experimental protocols.

2.1.4 – Acousto-optic flow measurement

The coupling of coherent light scattering and the acousto-optics effect has been recently exploited to measure blood flow (Adi Tsalach, 2015). This hybrid technique combines Ultra Sound and Diffused light for detecting changes is BF. The method could in principle disentangle deep from superficial blood flow.
Standard DCS is based on the fact that, when the sample’s scatterers move, the laser speckle pattern fluctuates in time, and the decorrelation time of the measured intensity depends on the average velocity of the scatterers. Another method for measuring the speed of the particles, that very recently has been shown to be equivalent to DCS, is Laser Doppler. The movement of RBC broadens the spectrum of light, because of the Doppler effect. This method works analysing the power spectrum of the scattered light, and not its autocorrelation function. The main drawback of Laser Doppler is that it lacks in depth resolution (as in the case of CW DCS) because the spectral broadening is a consequence of all the possible paths.

In the acousto-optic technique, also an ultrasound beam is sent into the tissue while illuminating the sample with the laser source. The US beam modulates the scatterers displacement, and consequently also the light intensity is modulated according to that. When the particles are moving, an additional Doppler shift occurs to the measured light. Thus, the spectral distribution of light, around the US frequency, carry information regarding the movement of the particles.

When a continuous US beam is used, the resulting broadening depends on all the path lengths, and thus is a cumulative effect of scatterers at all depths. On the other hand, if a pulsed US beam is used it is possible to modulate a specific slice of the sample at any given time (according to the delay with respect to the emission of the US pulse). Thus, one expects that different spectra (for various delay times) carry information about different depths. In fact, different US pulse delay correspond to different insonated layers. In reality, the spectral width associated to a specific depth is given by a sum of contributions of all the intermediate layers between the insonated one and the surface. For this reason, the spectral broadening associated to a specific layer must be deduced subtracting the overall spectral broadening of two neighbouring layers (two adjacent time delays). This subtraction gives rise to a local broadening that is associated with the local flow. The delay corresponds to the depth, while the local broadening corresponds to the flow rate (called Flow Index).

For now, the technique has been validated only with simulations and in-vitro experiments, thus still needs to be checked whether it is able to recover reliable BF measurements in an in-vivo experiment, and in particular in human studies.

From the previous Sections we see that an ideal depth-resolved Optical Blood Flow measurement technique still needs to be found. The techniques developed so far either make use of data fitting algorithms or to external manipulation for decoupling the superficial from the deeper flow, or need to be coupled with other methods (for instance US). On the other hand, TD DCS is an all-optical technique that potentially permits the depth-resolved measurements of Blood Flow. In the next section I am going to review the history of TD DCS, in order to understand what is the current state of the art of the technique, and what needs to be improved for permitting an in-vivo measurement on humans.

2.2 – Time Domain Diffuse Correlation Spectroscopy (TD DCS) techniques

2.2.1 – Pulsed Diffusive Wave Spectroscopy using non-linear optical gating

The first attempt to obtain a path length resolved information about scatterers motion is called Pulsed Diffusing Wave Spectroscopy, PDWS (A. G. Yodh, 1990). PDWS, instead of using a steady-state light source as standard DCS, uses a pulsed light source, as depicted in Figure
2.1. The pulses have a temporal duration $\delta t$ much smaller than their temporal separation $T$, and they have a carrier frequency $\nu_0$. With the use of a Beam Splitter (BS), each pulse is divided in a reference and sample branch. While the reference pulse passes through an optical delay stage, the sample pulse goes through the sample to be characterized. The pulse emerging from the Sample (S) is broadened in time (due to multiple scattering events) according to a specific distribution of path lengths $P(s)$, that depends on the optical properties of the sample to be characterized. Typically, the broadening of the pulse is larger than the width of the input pulse, and smaller than the repetition period $T$ (i.e. the time interval between two successive pulses). The scattered pulse is then recombined with the reference pulse in a Second Harmonic Generation (SHG) crystal. The generated second harmonic field is proportional to the product of the input fields, and before being detected is isolated from the first harmonic fields both spatially, with a pinhole (P), and spectrally, with a Second harmonic spectral filter (SF). The SH field is measured with a photomultiplier and then sent to an auto-correlator board to measure the intensity fluctuations on time scales $\sim \mu s$.

\[E_0(2\nu_0, t) \propto E_R E_S(t, s), \] \[\text{where } E_R \text{ is the reference field and } E_S(t, s) \text{ is the component of the scattered field arising from the path length } s. \]

From this, it is possible to show that the SH autocorrelation function $g_{1,SH}(\tau)$ is proportional to the product of the path length distribution $P(s)$ and the autocorrelation function of the scattered field $E_S(t, s)$. Thus, the SH autocorrelation $g_{1,SH}(\tau)$ is determined only by the path length $s$ fixed by the delay. By changing the time delay, different path lengths are probed. The measured autocorrelation function is given by the single path autocorrelation function (see Chapter 3), and it is not an average over $P(s)$.

The advantage of the technique is that is able to probe flows at different path lengths (and thus depths) separately. Additionally, since the temporal behaviour of the autocorrelation function does not depend on $P(s)$, is it possible to have a direct information about the mean square displacement of the scatterers $< \Delta r^2(\tau) >$, even for non-Brownian motion. This is particularly advantageous for probing particle motion at different time scales.

Figure 2-1: Scheme of the experimental setup used for pulsed DWS. The symbols used in the diagram are: BC, beam combiner; BS, beam splitter; L, lens; M, mirror; SHG: crystal for Second Harmonic Generation; PMT, photomultiplier tube; S, sample; P, Pinhole; SF, Second Harmonic spectral filter. Since the SH field is proportional to the product of the two input fields, it is like the sample pulse (upper branch) is gated by the reference pulse (lower branch). For this reason, only the path length $s$ (determined by the considered time delay) contributes to the SH field.

Since the reference pulse does not pass through the sample, it does not fluctuate in time. On the other hand, the electric field at the sample branch fluctuates in time, as a consequence of particle motion. The SH harmonic field is proportional to the product of the two fields, and it is determined only by the path length $s$, that depends on the time delay between the two branches. Thus, we can write $E_0(2\nu_0, t) \propto E_R E_S(t, s)$, where $E_R$ is the reference field and $E_S(t, s)$ is the component of the scattered field arising from the path length $s$. From this, it is possible to show that the SH autocorrelation function $g_{1,SH}(\tau)$ is proportional to the product of the path length distribution $P(s)$ and the autocorrelation function of the scattered field $E_S(t, s)$. Thus, the SH autocorrelation $g_{1,SH}(\tau)$ is determined only by the path length $s$ fixed by the delay. By changing the time delay, different path lengths are probed. The measured autocorrelation function is given by the single path autocorrelation function (see Chapter 3), and it is not an average over $P(s)$.
By measuring the mean intensity of the output field, is it also possible to retrieve the value of \( P(s) \) for the probed path lengths. This permits to compute the values of the optical parameters by fitting \( P(s) \) with respect to absorption and reduced scattering coefficients. The main disadvantage of the technique is that, since it is measured the SH field, it is necessary to use high intensities to obtain a sufficient signal. This is not possible in the case of in-vivo studies, because high power sources can damage biological tissues.

2.2.2 – TD DCS using software gating

Very recently, the technique arising from the combination of DCS and TD NIRS (named TD DCS) has been demonstrated experimentally (Sutin K., 2016). The technique is based on the parallel acquisition of the photons’ TOF and of the DCS fluctuations of the light diffused by the tissue. This is done with a Time Correlated Single Photon Counting (TCSPC) approach in which each photon is time tagged with two values: a fine time value (\( \sim ps \)) corresponding to the TOF in the tissue, and a coarse time value (\( \sim \mu s \)) corresponding to the absolute arrival time of the photon. The TOF is used for path length gating (and for evaluating the DTOF), while the absolute arrival time is used for evaluating the intensity fluctuations of the detected light. During the data analysis, before the photons are assigned to the gates according to their TOF, and then the autocorrelation function is computed using the absolute arrival time of the selected photons. In this way, it is possible to obtain autocorrelation functions corresponding to different path lengths in the tissue, and thus probe the scatterers motion at different depths.

The advantage of this software analysis is that several gates can be applied in parallel, one disadvantage is the computational complexity. The technique permits also the parallel computation of the DTOF starting from the TOF values, which can be used for TD NIRS for computing the static optical properties (i.e. absorption and scattering) of the medium.

DCS and TD NIRS have contradictory requirements regarding the properties of light sources to be used. In fact, as already discussed in Section 1.3, DCS needs a highly coherent light source to measure the laser speckles resulting from the interference of light. For this reason, typically DCS is performed using steady-state sources, which can easily have a coherence length longer than the span of photon path lengths in the tissue. On the other hand, TD NIRS uses pulsed light sources, with duration \( \sim 10 - 100 \text{ ps} \). Very short pulse duration is needed because too long input pulses do not permit an accurate evaluation of the optical parameters starting from the measured DTOF. The study shows that with the use of a high coherence pulsed light sources with duration \( \sim 100 \text{ ps} \), and narrow temporal gates with widths \( \sim 100 \text{ ps} \), it is possible to fulfil both the above-mentioned requirements on DCS and TD NIRS. In reality, I will show in Chapter 6 that using ultra-coherent sources it is possible to relax the requirement on the gate width and to use broader gates, with duration \( \sim 1 \text{ ns} \) (M. Pagliazzi, 2017).

Figure 2.2 shows the experimental setup used in the cited study. The setup uses a custom-made laser, with high peak power and picosecond duration. The light source is based on a two-stage laser Master Oscillator and Amplifier. As seed laser, it was used a Distributed Bragg Reflector laser in the near IR. By electrically gain switching the seed laser with a picosecond pulsed laser driver, light pulses with duration \( \sim 100 \text{ ps} \) are created. The pulse power is amplified using an electrically pumped taper diode amplifier and sent to the sample through an optical fiber. The light diffused by the sample is collected by another optical fiber and delivered to a red-enhanced Single Photon Avalanche Diode (SPAD) for detection. This sensor
is characterized by a high temporal resolution and quantum efficiency (i.e. photon detection probability). The resolution of the detector (and the shape of the light source) determines the Instrument Response Function (IRF) of the overall system, while the quantum efficiency affects the number of detected photons and thus the Signal to Noise Ratio (SNR). The output of the detector is sent, through a Time to Digital Converter card, to a FPGA board for recording the TOF of the detected photons. A coarser time value corresponding to the absolute arrival time of the photons (also called Correlation Time Tag) is also recorded in the FPGA, for computing the autocorrelation function. The time tags data of each photon are then sent to a computer for the computation of autocorrelation functions.

![Diagram of the experimental setup for TD DCS used in (Sutin K., 2016)]

It is important to note that, in this approach, the gating and the computation of the autocorrelation functions is performed in software. This permits to have more flexibility in the data analysis, since different gates can be applied in post processing. On the other hand, an analysis in software could be very time consuming (especially for high number of detected photons) and not permit a real-time measurement. Thus, a more efficient strategy could be the use of a hardware gating, in which the photons outside the gate are rejected switching off the detector, and a hardware correlator, in which the autocorrelation function is computed using specific devices called auto-correlators (see Chapter 4). Hardware gating has been already deployed successfully in TD NIRS, and will be discussed in more detail in the next Section. A brief discussion about software autocorrelation techniques can be found in Section 5.2.

2.3 – Fast-gated detectors for time domain measurements at small source-detection separation

In TD NIRS, the ability to extract information about deep tissues is mainly determined by the Dynamic Range (DR) of the used measurement system (Alberto Tosi, 2011). The DR is defined as the ratio between the minimum and maximum detectable intensities. While the maximum intensity is determined by the maximum count rate of the detection electronics, the minimum intensity is set by the background noise. In the case of functional brain activation for example,
a specific region of the brain is activated as a consequence of an external stimulus. The neuronal activation then triggers a hemodynamic response that carries important information regarding brain function. In this case, a big DR is needed because the number of late photons (the ones that go deep and actually reach the brain) is much smaller than the number of early photons.

When a short light pulse is injected in the tissue, the photons penetrate at a certain depth, which depends on the time delay between injection and detection of the photons. While the mean penetration depth increases for increasing TOF, it is possible to show that it does not depend on the source detector (SD) separation used. In addition, the number of detected photons increase for decreasing SD separation, at any time. For these reasons, it has been shown both theoretically and experimentally that the use of a short detector separation increases the penetration depth, spatial resolution and SNR of TD NIRS measurements (Pifferi, et al., 2008). For small SD separation, the number of early photons is very high compared to the number of late photons, and this is why standard TCSPC detection is not very effective in this case. In fact, in standard TCSPC, high count rates saturate the detection electronics and introduce distortions in the measured curves due to the fact that only one photon can be measured for each repetition of the laser. To overcome this, one diminishes the input power until the total count rate is sufficiently low. This method increases a lot the time necessary to reach a given SNR.

A method that has been developed quite recently for overcoming those limitations is the use of fast-gated single photon detectors, with fast transitions (approximately 200 ps) between the ON and OFF state. A fast-gated detector is a detector that is switched ON only in specific intervals of times. In this way, only photons arriving in a specific time window (i.e. belonging to a certain gate) are measured. In a small SD separation experiment, the use of such a detector permits to reject the high number of early photons and thus measure only the small number of late photons, the ones that carry information about deep tissue. Thus, since the early photons are not measured, the requirement on the maximum count rate become less stringent and it is possible to increase the injected power and, correspondingly, the resulting SNR and Dynamic Range.

This method could also be suitable for TD DCS measurements, since also TD DCS relies on single-photon measurement. Thus, the use of fast-gated single photon detector in TD DCS would permit for instance the use of small SD separation measurements, and all the related advantages regarding the higher DR/SNR and the better localization. Additionally, since only the photons belonging to the selected gate are measured, the computation of the autocorrelation function could be performed using a hardware correlator, which is a device available commercially, enabling a faster (and potentially real time) data analysis.

In the following, I am going to discuss a specific type of detector that is suitable for the above application, the Single Photon Avalanche Diode (SPAD). This detector can be operated in fast-gated mode and has the advantage that it does not get damaged when it is in the OFF state. A SPAD is a microelectronic circuit based on a p-n junction, reverse biased beyond the breakdown voltage. When a photon is absorbed, an electron avalanche is build up, and the rising edge of the avalanche’s current identify the arrival time of the photon with high (tens of picosecond) precision. When the photon is detected, a method for quenching (i.e. stopping) the avalanche must be used. This is done by reducing the bias voltage, and then resetting it to the original value in order to detect another photon. By rising and lowering the
bias voltage quickly, it is possible to obtain very short (~200 ps) raising and falling times. The detector is also insensible to big amounts of photons when it is in OFF state, because the avalanche cannot be sustained below the breakdown voltage. SPADs are characterized by high quantum efficiency (~20 % @ 800 nm), small dark count rate (~ 1 kcps) and high temporal resolution is ~35 ps. Since their active area is small, their collection efficiency is not very high. Figure 2.3 shows a typical temporal response of a SPAD detector.

![Figure 2.3: Typical temporal response of a SPAD, for a very narrow input laser pulse (Alberto Tosi, 2011).](image)

As it is possible to see in Figure 2.3, the temporal response of a SPAD can be divided in two regions. The sharp peak is due to photons that are absorbed in the active part (depleted region) of the detector, and thus the photo-generated electron ignites in a fast way the avalanche. The following tail is due to the photons that are absorbed in the neutral part. The corresponding electrons take more time to be trigger the avalanche since they need to diffuse from the neutral to the active area, for this reason the tail in the curve is called diffusive tail. The diffusion tail is the main limitation to the full suppression of early photons, because it is possible that an electron generated during the OFF state diffuses in the active area and then, when the detector is switched ON, ignites an avalanche. A second limitation of SPAD is their high after-pulse, which is a spurious signal arising from trapping and release of carriers in the detector. We will see in Chapter 6 that, for detectors with after-pulse, the autocorrelations are affected mainly at correlations times \( \tau \) shorter than ~1 μs, and thus the effect can be rejected by neglecting that part of the autocorrelation curve in the fit. We will see that the after-pulse makes more difficult the estimation the \( \beta \) parameter.

In the next Chapter I will review the basic theory of TD NIRS and DCS (Section 3.1 and 3.2) and I will discuss the theory of TD DCS (Section 3.3), trying to extend it to the case of non-ideal systems (i.e. detector with finite temporal resolution and light source pulse with finite temporal width).
Chapter 3 – Theory

In the first two Sections of this Chapter, I am going to present the theoretical background behind TD NIRS and DCS, i.e. Photon Diffusion Equation and Correlation Diffusion Equation. After that, in Section 3, I will propose a new model for TD DCS data analysis, that considers system non-ideality such that finite source pulse duration and finite temporal resolution of the detector. This new model has been developed to obtain more reliable estimation of the blood flow index (BFI) in real experiments, and have been validated (see Chapter 5) with Monte Carlo simulations.

3.1 – Time domain near infrared spectroscopy (TD NIRS) theory

3.1.1 – Photon Diffusion Equation

To interpret in a correct way the signals arising from NIRS, a model that permits to describe photon’s transport is required. One important quantity that describe photon transport in tissue is the Radiance \( L(r, t, \hat{\Omega}) \) \( [W/(sr \ cm^2)] \). The radiance is defined as the optical power per unit area travelling in the \( \hat{\Omega} \) direction, at position \( r \) and time \( t \) (Durduran T., 2010). This quantity is proportional to the square of the electric field’s absolute value. The Radiative Transport Equation (RTE) is an equation that describes the conservation of the radiance, which corresponds to the conservation of energy. Starting from the radiance, it is possible to compute the fluence rate \( \phi(r, t) \), defined as the optical power per unit surface exiting from an infinitesimal volume centred in \( r \), as follows:

\[
\phi(r, t) = \int_0^{4\pi} L(r, t, \hat{\Omega}) \, d\hat{\Omega} \quad [W/cm^2]
\]  

(3-1)

Another important physical quantity is the Photon Flux, which is defined as the vector sum of the Radiance along all directions:

\[
J(r, t) = \int_0^{4\pi} L(r, t, \hat{\Omega}) \hat{\Omega} \, d\hat{\Omega} \quad [W/cm^2]
\]  

(3-2)

With this definition, \( J(r, t) \cdot \hat{\Omega} \) is the power per unit area travelling in the \( \hat{\Omega} \) direction, at position \( r \) and time \( t \).

When suitable conditions are fulfilled, from RTE is it possible to derive a simpler equation that describes the behaviour of the fluence rate, and it is called Photon Diffusion Equation (PDE). In this case, we are modelling photon propagation in the diffusion limit (or diffusion approximation). In a medium with space-varying absorption and reduced scattering coefficients \( \mu_a(r) \) and \( \mu'_s(r) \), the general expression for the PDE is:

\[
\nabla \cdot (D(r)\nabla \phi(r, t)) - v \mu_a(r) \phi(r, t) - \frac{\partial \phi}{\partial t} = -vS(r, t)
\]  

(3-3)
Where \( v = c/n \) is the speed of light in the medium, \( D = 3v/(\mu'_s + \mu_a) \) is the photon Diffusion Coefficient, and \( S(r, t) \) is a Fluence source term. The main hypothesis for the validity of the PDE is that \( \mu'_s \gg \mu_a \) (reduced scattering much bigger than absorption coefficient). In addition to that, the radiance \( L \) needs to be nearly isotropic. When the assumption of the PDE are fulfilled, photon's trajectories in the medium can be seen as straight-line segments, which are interrupted either if the photon is absorbed or its direction is randomly changed. In addition, being the photon transport described by a diffusion equation, we can see the photon transport as a random walk. The length of each straight segment is approximately equal to the photon transport mean free path (or random walk step) \( l_{tr} = 1/\mu'_s \).

To use the PDE, it is necessary to verify that \( \mu'_s \) is much bigger than \( \mu_a \) (at least 10 times as a rule of thumb) and that photon propagation distance is much bigger than \( l_{tr} \). Additional hypothesis for the validity of the PDE are Source isotropy, slow temporal variations of Photon Flux, and rotational symmetry of the scattering. Close to boundaries, the Radiance can be not isotropic. To overcome this, it is necessary to introduce some Boundary Conditions (BC) to the problem, and to solve the PDE coupled with the selected BC. Some tissues in which the diffusion approximation can break are anisotropic tissues, like axon fibers bundles, and tissues with high absorption coefficient, for instance when a big amount of blood is present. Also, when small source detector separation is used, additional care must be used to apply PDE.

In most geometries of interest, the system under study present different boundaries. These boundaries need to be modelled with suitable Boundary Conditions to be inserted in the PDE. For instance, in a NIRS measurement in reflectance geometry, the light is delivered and collected from the tissue with optical fibers placed on the boundary between tissue and air. The basic assumption used to derive the BC is that photons that escape from the tissue never enter again. This means that the Photon Flux incoming into the tissue is given only by Fresnel reflections of the photons that hit the interface from inside. With this condition, and in the limit of the diffusion approximation, is it possible to show that the PDE show fulfil the so-called Robin (or partial Flux) boundary condition:

\[
\phi(r, t) = z_b n \cdot \nabla \phi(r, t), \text{ for } r \in \text{boundary}
\]

(3-4)

Where \( z_b = 2l_{tr}(1 + R_{eff})/(3(1 - R_{eff})) \), \( R_{eff} \) is the effective reflection coefficient of the boundary and \( n \) is a unit vector normal to the surface. This BC is difficult to impose to the PDE, and for this reason simpler BC has been developed to make the PDE easier to solve analytically. Expanding the Robin BC with a first order Taylor polynomial around the boundary, it is possible to derive the so called Extrapolated Zero BC:

\[
\phi(x, y, z = -z_b, t) = 0
\]

(3-5)

This condition is called Extrapolated Zero because it tells that the fluence rate needs to be zero on a plane outside the surface, distant \( z_b \) from the interface. This BC is particularly easy to impose to the PDE with the help of the method of images. From this is it possible to obtain easily the expression of the fluence rate in various geometries.

One the expression of the Fluence Rate is obtained (with the suitable geometry and BC), it is possible to obtain the Photon Flux at the surface by using the Fick’s law (M. S. Patterson, 1989):
\[ J(x, y, z = 0, t) = -D \nabla \phi |_{z=0} \]  

From this, one can compute the expression of the time-resolved Reflectance \( R \), which is defined as the number of photons reaching the surface per unit surface and per unit time:

\[ R(x, y, t) = |J(x, y, z = 0, t)| \]  

The reflectance is then the actual measurable quantity in a TD NIRS experiment.

The expression of the reflectance depends, in addition to the geometry chosen, also on the type of light source (See Chapter 1.1). Different kind of sources carry different amount of information regarding the measured tissue. As already pointed out, using a pulsed light source permits to obtain the maximum amount of information, because it permits to exploit an additional degree of freedom (time, or path length), that is not present when one uses steady-state sources. The simplest application of TD NIRS is the determination of the tissue optical properties, when the system is homogeneous. To do that, one compares the solution of the PDE with pulsed source term with the measured DTOF for a single source detector pair (see Chapter 1), and with some non-linear fit computes the reduced scattering and absorption coefficient of the tissue. When a inhomogeneous system is considered, is necessary to use in the data fitting the corresponding solution of the PDE, valid for inhomogeneous media. The human brain, for instance, can be modelled as a bilayer medium, where the upper layer is the scalp-skull and the lower layer is the brain. In this case with TD NIRS, knowing the thickness of the upper layer, is it possible to estimate accurately the values of the optical parameters of both upper and lower layers, without resorting to different source detector separations like in CW-NIRS. This method is particularly effective because it exploits the temporal information, one can use the small path length photons for estimating the upper layer properties and the big path length photons for the lower layer (Lucia Zucchelli, 2013).

3.1.1 - How we use TD NIRS theory for the data analysis

As we will see in Chapter 4, a TD DCS setup, additionally to measure the gated autocorrelation curves, it measures the DTOF curves.

The DTOF curve then can be used for the TD DCS data analysis. In fact, as we will see later, the theoretical expression of the gated autocorrelation functions depends on the path length probability within the tissue. This path length probability can be estimated using the theoretical expression of the reflectance. As the PDE depends on the absorption and reduced scattering coefficient of the tissue, also the expression of the reflectance will depend on those parameters, which are unknown. For this reason, in principle the easiest way to compute the path length probability is to use the measured DTOF, after deconvolution with the measured IRF. This method does not require the knowledge of the optical parameters of the tissue, and has the advantage to be independent on the boundary conditions (Diop & Lawrence, 2015). On the other hand, since the deconvolution operation is an ill-posed problem, complex regularization algorithms needs to be used to reject noise in the reconstruction. An easier method is to fit the measured DTOF with respect to the optical parameters against the theoretical expression of the reflectance (M. S. Patterson, 1989), convoluted with the measured IRF. For simplicity, in the fitting algorithm the reflectance is computed using a
semi-infinite homogenous model. In fact, our aim is not to estimate precisely the values of the optical parameters in all the points of the tissue, but simply to have an estimation of the overall reflectance function. For this reason, it is only necessary that the fit converges to the measured DTOF for estimating in a correct way the path length probability $P(s)$.

It is important to note that the measured DTOF can also be used for TD NIRS data analysis, for instance for evaluating Oxy- and Deoxy-haemoglobin concentration. From that it is possible to compute, in addition to the Blood Flow coming from TD DCS, other important haemodynamic quantities like oxygen saturation. Thus, in principle using additional wavelengths it is possible to estimate, in a depth resolved way, both the Blood Flow and the Oxygen Saturation.

### 3.2 – DCS theory

As already introduced in Chapter 1, DCS studies the fluctuation of the multiply scattered light emerging from a diffusive medium, to obtain information about scatterers motion. This technique originated from the DLS technique, which is valid in the single scattering case, and thus is applicable for optically thin samples. In this Section I will present the theory that describes single scattering autocorrelation functions called Dynamic Light Scattering (DLS), its extension to the multiple scattering case (DCS), and then I will present another model for describing the multiple scattering autocorrelation functions, the so-called Correlation Diffusion Equation (CDE), that simplifies data analysis for “standard” DCS. In those sections, for simplicity, effects arising from finite coherence length of the laser source will be neglected, a useful reference for those effects being (T. Bellini M. G., 1991).

#### 3.2.1 – Single scattering limit: dynamic light scattering

In a DLS experiment, a steady state and highly coherent laser shines an optically thin sample (Zhou, 2007). Light is scattered by the sample, and it is detected at a certain angle $\theta$ relative to the direction of the input light beam. Light intensity fluctuations are recorded, and then the autocorrelation function of the intensity is computed and analysed. A typical DLS setup is depicted in Figure 3.1.

![Figure 3-1: Typical DLS experimental setup (Durduran T., 2010).](image)

The measured quantity in a DLS experiment is the normalized intensity autocorrelation function, computed as:

$$g_2(\tau) = \frac{<I(t)I(t+\tau)>}{<I(t)^2>} \quad (3-8)$$
Where $I(t)$ is the detected intensity and $< >$ denotes a *temporal* average.

Later on, we will see how this quantity is related with the normalized electric field ($E(t)$) autocorrelation function:

$$g_1(\tau) = \frac{<E(t)E^*(t+\tau)>}{<E(t)E^*(t)>} \quad (3-9)$$

In a DLS experiment, if the light source is stationary and monochromatic, and one assumes that the particles are randomly positioned, and their motion is uncorrelated, it is possible to show that the electric field autocorrelation function $g_1(\tau)$ can be expressed as:

$$g_1(\tau) = e^{i\omega \tau} <e^{i\varphi \Delta r(\tau)}> \quad (3-10)$$

Where $\omega$ is the angular frequency on the light source, $\tau$ is the correlation time, $q = k_{\text{out}} - k_{\text{in}}$ is the difference between the wave vectors ($k = \frac{2\pi}{\lambda} n$) before and after the scattering event, $\Delta r(\tau)$ is the displacement of the scatterers at time $\tau$ and $<>$ denotes an *ensamble* average (average over all the possible realizations of the sample).

If one then assumes that the particles move in a random way, we expect that the particles displacement $\Delta r(\tau)$ follows a Gaussian probability distribution. In this case, using this distribution, we can explicitly compute the average present in the right-hand side of the last equation and obtain:

$$g_1(\tau) = e^{i\omega \tau} e^{-\frac{1}{6}q^2<\Delta r(\tau)^2>} \quad (3-11)$$

Where it is possible to use the expression $q = 2k_0\sin(\frac{\varphi}{2})$ for the momentum transfer (being $k_0 = \frac{2\pi}{\lambda} n$ the incident wave vector and $\theta$ the angle between incident and scattered wave-vectors), and $<\Delta r(\tau)^2>$ is the mean square displacement of the scatterers at time $\tau$. Due to particle motion, the photon paths undergo slight changes, that cause a dephasing of the detected electric fields (due to slight changes in path length), and thus a decay of its autocorrelation function. Because the autocorrelation function decays to zero for displacements $\Delta r$ of the order of $\lambda$, DLS can probe scatters motion on the scale of a wavelength.

Now we note that the theoretical model presented so far refers to $g_1$, the electric field autocorrelation, while it is possible to measure directly only $g_2$, the intensity autocorrelation. On the other hand, it is possible to prove that for sources which are stationary Gaussian sources and for ergodic samples\(^2\) (like for instance particles in suspension and biological tissues) the following relation, in the limit of infinite coherence length, holds:

$$g_2(\tau) = 1 + \beta |g_1(\tau)|^2 \quad (3-12)$$

Where $\beta$ is a parameter that depends on the detection optics, and is proportional to the inverse of the number of detected modes. The last relation is called Siegert relation and it

---

\(^2\) In an ergodic system, the ensemble and temporal average of any quantity give the same result.
permits us to estimate $g_1(\tau)$. $\beta$ can be determined experimentally as the intercept of $g_2(\tau)$ for $\tau = 0$, minus one. In the case of unpolarised light and for detection with a single mode fiber and typical CW sources (i.e. highly coherent) we have $\beta \sim 0.5$.

Regarding to the properties of the scatterers motion, typically two models are used: Brownian motion or Random flow. In the case of Brownian motion, $<\Delta r(\tau)^2> = 6D_B \tau$, where $D_B [cm^2/s]$ is the particle diffusion coefficient. In the case of random flow, $<\Delta r(\tau)^2> = <V^2> \tau^2$, where $<V^2>$ is the second moment of particle speed distribution. For biological tissues it has been show that the Brownian model fits better the experimental data, even though the reason of this Brownian-like signal is still not clear.

3.2.2 – Multiple scattering limit: DCS

When we consider an optically thick sample, photons undergo multiple scattering events in the tissue, thus the dephasing of the electric field (and the corresponding decay of the autocorrelation function) will be a cumulative effect of all the scattering events along the photon path (Zhou, 2007). To quantify this, one before considers a single path of the photons within the tissue, as depicted in Figure 3.2.

![Figure 3-2: multiple scattering events in a photon’s single path, for the calculation of the electric field autocorrelation function in the case of an optically thick medium (Durduran T., 2010).](image)

When one considers multiple scattering events, the electric field autocorrelation function relative to a single photon path can be written as:

$$g_{1, \text{single path}}(\tau) = e^{i\omega \tau} < \prod_{i=1}^{N} e^{iq_i \Delta r_i(\tau)}>$$

(3-13)

Where $q_i$ and $\Delta r_i(\tau)$ are the momentum transfers and particle displacement at the $i$th scattering event along the given path, and $N$ is the total number of scattering events along the path. In a homogeneous medium, if we assume each scattering event is independent and the scatterers displacements are uncorrelated, we can compute the average using a Gaussian distribution for the scatterers displacement (as in the single scattering case) obtaining:

$$g_{1, \text{single path}}(\tau) = e^{i\omega \tau} \prod_{i=1}^{N} e^{-\frac{1}{6}q_i^2<\Delta r(\tau)^2>} = e^{i\omega \tau} e^{-\frac{1}{6}<\Delta r(\tau)^2> \sum_{i=1}^{N} q_i^2}$$

(3-14)
Where we can express the momentum transfer at the \( i \)-esim scattering event as \( q_i^2 = 2k_0^2(1 - \cos(\theta_i)) \).

If we define the so-called adimensional momentum transfer as \( Y = \sum_{i=1}^{N}(1 - \cos(\theta_i)) \), we can write:

\[
g_{1,\text{single path}}(\tau) = e^{-\frac{1}{2}k_0^2Y<\Delta r(\tau)^2>}
\]

It is then possible to re-express \( Y \) as:

\[
Y = \sum_{i=1}^{N}(1 - \cos(\theta_i)) = N(1 - \overline{\cos(\theta)})
\]

Where \( \overline{\cos(\theta)} \) is the average of the cosine of the scattering angle along the \( N \) scattering events. In a diffusive medium, where \( N \) is sufficiently high, we can substitute this average with the ensemble average \( <\cos(\theta)> = g \), where \( g \) is the so-called anisotropy factor. Doing this one obtains \( Y \cong N(1 - g) = \mu's \). Substituting the last expression in the single path autocorrelation function, and supposing Brownian motion of the scatterers, we obtain:

\[
g_{1,\text{single path}}(\tau) = e^{-k_s\tau}
\]

Where the constant \( k \), the autocorrelation decay rate per unit path length, is defined as:

\[
k = 2\mu'k_0^2\alpha D_B
\]

And \( \alpha \) is the fraction of moving scatterers in the medium.

In a “standard” DCS experiment, since one uses a steady-state source, all the path lengths contribute to the measured autocorrelation. Thus, the overall autocorrelation function will be a weighted average of the single path autocorrelation, over the path length probability \( P(s) \), estimated with \( R_{th}(s) \):

\[
g_1(\tau) = \int_0^\infty R_{th}(s) \exp(-k_s\tau) \, ds
\]

We note that typically in an in-vivo experiment both \( \alpha \) and \( D_B \) are unknown, but this is not a problem because one can define a Blood Flow Index \( BFI = \alpha D_B \) to quantify the haemodynamic response\(^3\). The units of this BFI \([cm^2/s]\) are different than the standard units for Blood Flow \([\frac{ml}{min}/100\, g]\), however different studies (see Section 1.2) showed that the BFI measured with DCS correlates well with several other Blood Flow measurement techniques, like Doppler Ultrasound and MRI. From the previous equation we see that, in a “standard” DCS experiment, all the path lengths contribute to the measured autocorrelation and to its decay rate, consequently the measured BFI will contain contributions from all the depths of the medium.

\(^3\) On the other hand, on liquid phantoms one sets \( \alpha = 1 \) and so \( BFI = D_B \).
3.2.3 – Correlation Diffusion Equation

In a very similar way than PDE, it is possible to show (Durduran T., 2010) that the un-normalized electric field autocorrelation:

\[ G_1(\mathbf{r}, \tau) = \langle \mathbf{E}^*(\mathbf{r}, t) \cdot \mathbf{E}(\mathbf{r}, t + \tau) \rangle \]  

(3-20)

follows the following Correlation Diffusion Equation (CDE):

\[
\nabla \cdot (D(\mathbf{r}) \nabla G_1(\mathbf{r}, \tau)) - (\nu \mu_a(\mathbf{r}) + \frac{\alpha}{3} \nu k_0^2 \mu_s' < \Delta r(\tau)^2 >)G_1(\mathbf{r}, \tau) = -\nu S(\mathbf{r})
\]

(3-21)

Where \( < \Delta r(\tau)^2 > \) is the mean square displacement of the scatterers at time \( \tau \), \( \alpha \) is the fraction of moving scatterers and \( S(\mathbf{r}) \) is an isotropic, steady-state source term. All the other quantities have the same meaning of their fluence rate counterpart. Note that the CDE equation is formally equivalent to the PDE equation, except for the fact that here it is present an additional “absorption” term due to the motion \( < \Delta r(\tau)^2 > \) of the scatterers. Also, the Boundary Conditions are completely analogous to their PDE counterpart. For instance, the Extrapolated Boundary Condition for the autocorrelation is:

\[ G_1(x, y, z = -z_b, \tau) = 0 \]

(3-22)

Note that if one lets \( \tau \to 0 \) in the CDE, we obtain the steady-state case of the PDE. For all these reasons, the solution of the CDE is particularly simple if one already knows the corresponding solutions of the PDE. For instance, in the case of a semi-infinite homogenous medium, assuming extrapolated BC, the solution of the CDE is:

\[
G_1(\rho, z, \tau) = \frac{\nu}{4\pi D} [e^{-K(\tau)r_1/r_1} - e^{-K(\tau)r_b/r_b}]
\]

(3-23)

Where:

\[
\begin{aligned}
K(\tau) &= \sqrt{\frac{\nu}{D} (\mu_a(\mathbf{r}) + \frac{\alpha}{3} \nu k_0^2 \mu_s' < \Delta r(\tau)^2 >)} \\
r_1 &= \sqrt{(z - l_{tr})^2 + \rho^2} \\
r_b &= \sqrt{(z + 2z_b + l_{tr})^2 + \rho^2}
\end{aligned}
\]

(3-24)

One then obtains the normalized electric field autocorrelation simply as \( g_1(\rho, z, \tau) = G_1(\rho, z, \tau)/G_1(\rho, z, \tau = 0) \). Thus, we can see that the autocorrelation function carry information about scatterers motion because \( G_1 \) depends on the mean square displacement \( < \Delta r(\tau)^2 > \). As in the previous Subsections 3.2.1-2, one can use the Siegert relation to compute the \( g_1 \) function from the measured \( g_2 \).

3.2.4 - How we use DCS theory for the data analysis
In my experimental activity, instead of a steady-state source it has been used a pulsed source. This permits to compute path length resolved autocorrelation functions, i.e. with temporal gating only certain path lengths are selected and contribute to the measured autocorrelation.

In our TD DCS experiments, we have used three different kinds of temporal gates:

- Narrow gates, width \( \sim 100 \text{ ps} \)
- Broad gates, width \( \sim 500 \text{ ps} - 2 \text{ ns} \)
- Ungated acquisition

Due to requirements on the coherence of the pulsed laser source, for now in TD DCS only the narrow gate approach has been used (Sutin K., 2016). It is called narrow because its duration is much shorter that the duration of the DTOF curve. Since the gate width is very short, the path length distribution in the gate is very narrow, so the autocorrelation function will be approximately given by the single path autocorrelation function, a single exponential. For this reason, in data fitting we have used the single path autocorrelation function, as other authors (Sutin K., 2016). Since the SNR in a DCS experiment depends on the detected intensity (Chao Zhou, 2006), a narrow gate approach offers a limited SNR due to the low detected photon flux.

The second approach is the use of broad gates. In this case, the path length distribution is not narrow anymore, thus the single path autocorrelation function is not anymore a good model. For this reason, as we will see in Section 3.3, in the data fitting we have used the integral expression of the autocorrelation function, in which we weight the single path autocorrelation function over the path length probability \( P(s) \). The only care we need to take is that, in this integral over the path lengths, only the path lengths belonging to the gate \( s \in [a,b] \) needs to be considered (M. Pagliazzi, 2017). The optical parameters to be used in the \( P(s) \) have been estimated with TD NIRS. We note that in this case the CDE is not correct because it supposes that all the path lengths contribute to the measured autocorrelation function, and not only the path lengths belonging to the gate. We note that the use of broad gates permits a larger SNR due to the higher photon flux, but it is possible only if the pulsed source is highly coherent.

The third approach is the use of ungated acquisition. This way of acquisition corresponds to not applying any gate to the photon path lengths, thus collecting the whole DTOF curve. For this reason, all the paths lengths will contribute to the autocorrelation. In this case, we have used for data fitting the solution of the CDE, with optical parameters evaluated using TD NIRS. In principle it is also possible to use also the integral expression of the autocorrelation function, in which one integrates over all the possible path lengths (i.e. \( s \in [0,\infty) \)), however since the solution of the CDE does not contain an integral, it is less affected by computational errors and thus permits a more robust data fitting. Regarding to SNR and coherence requirements same consideration as broad gates acquisition apply.

3.3 – Effect of Instrument Response Function (IRF) on TD DCS

The aim of a TD DCS experiment is to extract a depth-resolved Blood Flow Index (BFI) using time gated intensity autocorrelation functions. TD DCS relies on the fact that, by selecting different photon time of flights (TOF) and thus different path lengths undergone in the medium, photons probe different depths. Using an ideal system (ideal detector and input
pulse with zero width), it is possible to measure and select accurately the path lengths belonging to a certain temporal gate, and thus $D_B$ can be estimated in a precise way from the $g_1(\tau)$ decay rate. On the other hand, a non-ideal system (noisy detector and finite pulse width) can be treated as Linear Time Invariant (LTI) and therefore characterized only by measuring its Instrument Response Function (IRF) which, differently from the ideal case, has a certain temporal width (i.e. is not a delta of Dirac). In this case, perfect path length selection is not possible, thus the measured $g_1(\tau)$ will contain spurious contributions from path lengths in the proximity of the gate. This can give rise to a wrong estimation of the BFI even for a homogenous medium, because the $g_1(\tau)$ decay rate is different than expected. In addition to this, for a medium with non-homogeneous flow, photons that probe shorter path lengths can be wrongly assigned to gates corresponding to longer path lengths, or vice versa. Thus, the retrieved flow value for a given depth could be contaminated by the flow values at different depths. For those reasons, a correct modelling of the effects arising from the IRF on gated autocorrelation functions is strongly needed.

### 3.3.1 - Ideal TD DCS experiment

In an ideal DCS experiment, in the case of ungated acquisition, the electric field autocorrelation function can be expressed as:

$$g_1(\tau) = \int_0^\infty R_{th}(s) \exp(-ks\tau) ds.$$  \hspace{1cm} (3-25)

The quantity $R_{th}(s)ds$ can be interpreted as the probability of detecting a photon that underwent a path length equal to $[s, s + ds]$ in the medium. It depends on the absorption and reduced scattering coefficients, on the geometry, and on the way in which the source and the detector are placed (i.e. the boundary conditions).

When we make a Time Resolved experiment, we estimate the path length $s$ starting from the photon’s Time of Flight (TOF), i.e. the time that a photon spends into the tissue. If the medium considered has uniform refractive index $n$, we can write $s = vt$ where $v = c/n$ is the speed of light in the medium, and $t$ is the ideal photon Time of Flight (TOF). The TOF can be measured as the difference between light pulse emission and detection times (by using pulsed lasers and fast single photon detectors coupled with dedicated timing electronics). In this section I will analyse the effect that a non-ideal detection system has on gated autocorrelation functions in a realistic TD DCS experiment.

In an ideal TD DCS experiment, in which the source light pulses have a negligible temporal duration, and the detection system has infinite resolution, we are able to accurately estimate the path lengths of the photons. In this case if we select, by means of a time gate, the TOF counterpart of a certain path length interval $s \in [a, b]$, the (gated) autocorrelation can be written starting from (3-1) by considering only the path lengths within the given gate:

$$g_1(\tau) = \int_a^b R_{th}(s) \exp(-ks\tau) ds$$  \hspace{1cm} (3-26)

### 3.3.2 - Realistic TD DCS experiment: a model
In the real case, the source light pulses have always a certain finite duration, and the detection system has a temporal resolution that is also finite. To quantify this, we can model the system as Linear Time Invariant (LTI). In this way, the system can be characterized by its Instrument Response Function (IRF).

In the ideal case, the IRF is a Dirac delta function in time, centred in the emission time of the pulse (time that corresponds to the zero path length). Experimentally, it is possible to measure the IRF by facing the input and output fibers, separated by a thin film (for intensity attenuation and mode mixing), and by measuring the resulting distribution of the time of detection of the photons.

The IRF interpreted as a probability distribution

In this part, I will try to introduce the effect of a non delta of Dirac IRF function in the gated autocorrelation functions that are measured in a real TD DCS experiment.

First, we note that due to the IRF there is a difference between the real path length $s'$ (the path length that a photon actually underwent into the tissue) and the measured path length $s_0$ (the path length that we estimate from the measurement, using the measured TOF). In fact, the IRF alters the probability of detecting a photon that underwent a real path length $s'$ in a time bin that corresponds to a measured path length $s_0$ exactly equal to $s'$. For now on, I will assume that the IRF has been rescaled in time such that its peak is at $t = 0$. This will be useful later, because the time at which the probability of emission of a photon is maximum can be estimated with the temporal position of the IRF peak (that will be denoted with $t_0$).

An interesting quantity is the conditional probability that a photon that underwent a real path length $s'$ has been assigned by the system to a time bin that corresponds to an apparent path length $s_0$; we denote this quantity with $P(s_0|s')$. If we normalize the IRF such that its area is equal to one (and because we have rescaled it such that its maximum is at $t = 0$), we can link this probability with the IRF as follows:

$$P(s_0|s') = IRF(s_0 - s') = \frac{P(s_0 \cap s')}{P(s')},$$

(3-27)

Where $P(s_0 \cap s')$ is the joint probability that a photon has been detected in an apparent path length $s_0$ and has been generated with a real path length $s'$. In the second step, we have used the definition of conditional probability (Ross, 2014). If $R_{th}$ is normalized such that its area is equal to one, we can write $P(s') = R_{th}(s')$. We note that, since the system is time invariant, the conditional probability depends only on the difference between the real and the measured path lengths, and not on the value of the “starting” path length $s'$, see Figure 3.1.

At this point, we note that the average that we carry out to compute the measured autocorrelation function is a temporal average, while the theoretical expressions for the autocorrelation functions is often derived using an ensemble average (i.e. averaging over a certain probability distribution) (Bruce J. Berne, 2000).

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4 In the literature, some authors estimate this initial time with the time at which the rising side of the IRF emerges from noise.
In an ideal system, the probability distribution depends only on the real path length, thus we use $P(s')$. In a real system, the IRF makes some confusion between $s'$ and $s_0$, and thus we need to use the joint probability $P(s_0 \cap s')$. With the use of the previous equation, we can rewrite this probability as:

$$P(s_0 \cap s') = P(s_0 | s') P(s') = IRF(s_0 - s') R_{th}(s')$$  \hspace{1cm} (3-28)$$

In this way, we have related the joint probability with either measured quantities (IRF) or known quantities ($R_{th}$, if the optical parameters are known or fitted using the DTOF and the IRF).

Since the single path autocorrelation decay rate depends on the real path length $s'$ (because the decay rate is proportional to the number of scattering events along the path), we can write the infinitesimal contribution to the autocorrelation function (corresponding to photons with $s' \in [s', s' + ds']$, detected within a gate $s_0 \in [s_0, s_0 + ds_0]$) as:

$$dg_1 = P(s_0 \cap s') \exp(-ks' \tau) ds_0 ds' = IRF(s_0 - s') R_{th}(s') \exp(-ks' \tau) ds_0 ds'$$  \hspace{1cm} (3-29)$$

We need to integrate this expression over all the possible real and measured path lengths to get the overall autocorrelation function. One important point is that, since we are applying the gate according to the measured path length (and not on the real), we need to impose $s_0 \in [a, b]$. On the other hand, a photon with real path length $s'$ can be mapped into the gate also if it was coming from outside the gate (the only constrain is that it should have a path length larger than the SD separation $\rho$), thus we have $s' \in [\rho, \infty]$. Thus, we get:

$$g_1(\tau) = \int_{\rho}^{\infty} ds' \int_{a}^{b} ds_0 IRF(s_0 - s') R_{th}(s') \exp(-ks' \tau)$$  \hspace{1cm} (3-30)$$

We can see that the only quantity depending on $s_0$ is the IRF, thus we can write the final expression of the gated autocorrelation as:

$$g_1(\tau) = \int_{\rho}^{\infty} \theta(s') R_{th}(s') \exp(-ks' \tau) \, ds'$$  \hspace{1cm} (3-31)$$
Where we have defined the integral function $\theta(s')$ as:

$$
\theta(s') \doteq \int_a^b IRF(s_0 - s')ds_0
$$

Comparing the last definition with the expression of the conditional probability, we can see that $\theta(s')$ can be interpreted as the probability that a photon with real path length $s'$ is assigned to the gate $[a, b]$. Thus, the contribution of $s'$ to the autocorrelation does not depend only on the probability that a photon is generated with path length equal to $s'$, but depends also on the probability that a photon with path length $s'$ is mapped into the gate (that is described by the function $\theta(s')$). This function, since depends only on the measured IRF, can be readily estimated with a numerical integration.

We note that, in the expression $g_1(\tau)$, the time gating is included in the definition of the $\theta(s')$ function. To have the autocorrelation function normalized ($\lim_{\tau \to 0} g_1(\tau) = 1$) we need to impose:

$$
\int_\rho^\infty \theta(s')R_{th}(s')ds' = 1
$$

In the following we are going to discuss some cases of the equation (3-7): ideal system (delta of Dirac IRF), ungated acquisition and narrow gates.

3.3.2 – Some particular cases

If we consider equation (3.8), we can see that we can transform the limits of integration of the integral from finite (between $a$ and $b$) to infinite by writing:

$$
\theta(s') = \int_{-\infty}^{+\infty} \chi_{[a,b]}(s_0)IRF(s_0 - s')ds_0
$$

Where we have introduced a rectangular function $\chi_{[a,b]}(s_0)$, which is equal to one for $s_0 \in [a, b]$, zero elsewhere, and that will be called $gating function$ in the following. From eq. (3-10), one can see that the Theta function is expressed as a cross-correlation of the gating function and of the IRF\(^5\).

**Ideal system**

In the case of ideal system, the IRF can be described as a delta of Dirac centred in the origin: $IRF(x) = \delta(x)$. If we introduce this expression of the IRF in eq. (3-10), we get:

$$
\theta(s') = \int_{-\infty}^{+\infty} \chi_{[a,b]}(s_0)\delta(s_0 - s')ds_0 = \chi_{[a,b]}(s')
$$

Inserting (3-35) in (3-31), we can see that we recover the usual expression of the autocorrelation function (3-26).

---

\(^5\)Thus, the IRF can be seen as a correlation filter. Furthermore, using the relation between cross-correlation and convolution, the Theta function can be expressed as the convolution between the gating function and the $time-reversed$ version of the IRF.
Thus, we can see that in the case of delta of Dirac IRF, the Theta function has the same shape of the gating function (i.e. a rectangle). On the other hand, when the IRF has a certain width, the Theta function gets smoothed (see Footnote 5) and so the $g_1(\tau)$ curve gets distorted. For this reason, we can interpret the $\theta(s')$ function as an effective gating function, that gives the path lengths that contribute to the autocorrelation function $g_1(\tau)$. The shape of the effective gating function $\theta(s')$ is then the net result of two opposite effects: a sharpening effect (due to the temporal gating) and a broadening effect (due to the IRF).

**Ungated acquisition**

The case of ungated acquisition corresponds to a gate that has a duration comparable with the total duration of the DTOF curve. In this case, it is important to note that, while the real path length $s'$ needs always to be positive, the measured path length $s_0$ in certain cases can also become negative. In fact, the photons that have a real TOF very close to time zero (the peak of the IRF) have a certain probability to be mapped slightly before the peak of the IRF (and thus to negative times). For this reason, to correlate all the photons, we need to choose a gate that spans the whole DTOF curve, and this can be described mathematically as $(a, b) = (-\infty, \infty)$. Thus, in this case we have, using (3-32):

$$\theta(s') = \int_{-\infty}^{+\infty} \delta(s_0 - \bar{s})ds_0 = 1$$ (3-36)

In which we have used the normalization property of the IRF.

Thus, in the case of ungated acquisition, even for a IRF with a non-zero width, the effective gating function is equal to one (as in the ideal case), and so the autocorrelation $g_1(\tau)$ is still described by equation (3-1). In fact, since in ungated acquisition we are collecting all the photons, there are no photons that can go outside or inside the gate due to the IRF, and thus we do not expect any deviation of the autocorrelation curve from the ideal expression because we are correlating the “right” photons. Further study is necessary to understand if ungated TD DCS measurement is equivalent to a CW DCS measurement.

**Narrow gates**

Another interesting case is the one of narrow gates, i.e. temporal gates that have a width shorter than the time scale at which the DTOF varies. For typical tissues, this corresponds to a duration smaller than $\sim 100 \text{ ps}$. A narrow gate can be described by its central path length $\bar{s} = \frac{a+b}{2}$ and its width $\Delta s = b - a$. In the limit of very narrow gates (i.e. $\Delta s \to 0$, or equivalently $a, b \to \bar{s}$) we can approximate the gating function as:

$$\lim_{\Delta s \to 0} \chi_{[a,b]}(s_0) = \delta(s_0 - \bar{s})$$ (3-37)

In this case, using (3-10) we can write the Theta function as:

$$\theta(s') = \int_{-\infty}^{+\infty} \delta(s_0 - \bar{s}) IRF(s_0 - s')ds_0 = IRF(\bar{s} - s')$$ (3-38)

And inserting this expression in (3-7) we get finally:
\[ g_1(\tau) = \int_0^\infty IRF(\bar{s} - s')R_{th}(s') \exp(-ks'\tau) ds' \]  

(3-39)

While, for a delta of Dirac IRF, the autocorrelation is given by a single exponential function \( e^{-k\bar{s}\tau} \), when we add the IRF also the neighbouring path lengths contribute to the autocorrelation function, and thus \( g_1(\tau) \) gets distorted. In the analysis carried out in previous works (Sutin K., 2016) this effect was neglected. Due to this, it is not possible to define anymore a narrow gate autocorrelation decay rate \((ks)\), because also path lengths \( s' \neq \bar{s} \) contribute to the \( g_1(\tau) \). We will see in Chapter 5 that a way to bypass the problem is to directly compute the \( D_B \) with a fit of eq. (3-40).

In Section 4.4 I will present Monte Carlo simulations that have been used to validate this model. The use of the proposed model could allow one to recover more accurate values of the BFI also in real experiments, by compensating for the \( g_1(\tau) \) distortions that are coming by the effect of the IRF. Another important step should be to verify the model with the help of real experiments.

---

6 It is possible to verify it inserting the ideal expression of the IRF \((IRF(x) = \delta(x))\) in eq. (3-39) and neglecting the constant factors.
Chapter 4 – Monte Carlo simulations

4.1 – The Monte Carlo method

Monte Carlo simulations are a powerful method for modelling transport in tissues. In fact, Diffusion theory is a fast method for modelling the light transport in this kind of materials, but in certain conditions it does not give accurate results, for instance close to the sources or to the boundaries, or when absorption is comparable with scattering (Jaques, 2011). Furthermore, as we will see next, it permits to obtain in a relatively simple way several physical quantities, for instance the DTOF and the autocorrelation function. Thus, Monte Carlo method is useful both for its accuracy and for its versatility.

In a Monte Carlo simulation, photons are injected into the tissue with a given initial position \((x, y, z)\) and direction, specified with the use of directional cosines \((\alpha, \beta, \gamma)\), see Figure 4.1. After the launch, the distance that a photon travels before it interacts is selected using a random number \(\xi \in [0,1]\), and the (total) attenuation coefficient \(\mu_{\text{tot}} = \mu_a + \mu_s\). This selection, as I will describe later, is made using a random sampling method called “basic Monte Carlo sampling”. After the interaction, a “weight” of the photon (that initially is set to 1) is reduced according to the absorption coefficient. The remaining part of the weight is redirected according to a function called ‘phase function’, that describes the angular dependence of the scattering. After this, the photon again moves a random distance and the cycle is repeated.

![Figure 4.1: Schematic representation of a Monte Carlo simulation in reflectance geometry. Each photon is injected into the tissue with a certain initial position and angle and does several steps into the medium. These steps determine the path of the photon. Each photon can be absorbed by the medium (red arrow with final cross) or re-emitted from the surface (black arrow). The medium can have also not homogenous optical properties to simulate real biological tissue.](image)

It is possible to include in the simulation also refraction at refractive-index-mismatch boundaries and changes in the local optical properties. Some others interesting physical quantities such as the heat generated into the tissue can be calculated, but they will not be considered in my work.
Now I am going to discuss the method that Monte Carlo uses for sampling probability densities (the ‘basic Monte Carlo sampling’), that have been useful, as we will see later, for making simulations of a TD DCS experiments in realistic conditions.

4.1.1 – Basic Monte Carlo sampling

In a Monte Carlo simulation, some important quantities regarding the photon propagation such as step size, scattering angle and reflection/refraction by boundaries are determined by random sampling (Jaques, 2011). Given a generic Probability Density Function \( p(x) \), its Cumulative Distribution Function \( F(x_1) \), evaluated a particular value \( x_1 \) of the random variable \( x \) is defined as:

\[
F(x_1) = \int_{-\infty}^{x_1} p(x)dx
\]  

(4-1)

To sample the random variable \( x \), before a random number \( \xi \in [0,1] \) is generated, then \( x_1 \) is computed according to:

\[
\xi = F(x_1)
\]  

(4-2)

It is just necessary to solve Equation (4.2) with respect to \( x_1 \). Figure 4.2 shows graphically the use of the method.

Figure 4-2: Basic Monte Carlo sampling of a generic random variable \( x \), given its probability density function \( p(x) \), using a random number \( \xi \) and the cumulative distribution function \( F(x) \).

If a series of values of \( \xi \) is generated, we will have a corresponding series of \( x_1 \). This method can be applied to any random variable \( x \), and for any (normalizable) probability density function. The random variables used here are the step size, the scattering angle and the reflection by the surface of the medium. As I will show later, we have applied this method to implement in the Monte Carlo simulation the effects of a finite temporal resolution in the measurement of the TOF of the photons. This effect can be described by the temporal response of the detector, which is often called Instrument Response Function (IRF).
4.1.2 – ICFO program: MBio

For my Monte Carlo simulations, I have used a program called MBio. MBio is a program developed and continuously improved in the Medical Optics group at ICFO. It is based on the MCML program (Wang L., 1995) and permits the use of several kinds of geometries, non-homogeneous optical properties distribution and permits the use of different types of light sources (collimated, isotropic, ...). The simulation is launched with the use of an input file. In the input file, the user needs to select the parameters of the simulations. In particular, the input files need to contain:

- The grid geometry (the voxel division of the medium)
- The light source (collimated, isotropic etc.)
- Time division for TRS (time bins)
- Materials (with their optical properties)
- Shape of the materials (cubic, spherical)
- Detectors (cubic, cylindrical)
- Flow properties ($D_B$)

After the input file is written, the simulation is launched simply selecting the number of photons that needs to be simulated. Once the simulation is terminated, several output files are generated:

- The echo file (a summary of the used parameters)
- The absorption file (absorption map)
- The autocorrelation files
- The history file (see later)
- The TRS file (that contains the DTOF with the given time bins)

The history file contains additional information, that in our case (as we will see in section 4.2) are necessary to build the gated autocorrelation function. For each detected photon packet, it is stored in the history file:

- The position of detection
- The arrival weight ($W$)
- The path length in each medium ($s$)

The position of detection is present in the case of a detector that is not point-like, and tells in which part of the detector the photon is measured, while the arrival weight gives the residual weight of the photon packet when it reaches the detector.

The first step of my work on this simulation have been to understand how to build the DTOF and the gated autocorrelation functions from these files. In fact, in the most recent version, MBio was able to compute only ungated autocorrelation functions (i.e. by considering the whole span of photon path lengths). Another problem that I had to face was that the DTOF, in MBio, is saved using logarithmic time binning, and for our application it was more useful to have linear time binning. In the next chapter I will describe how I faced these issues and the results of TD DCS simulations. Then, I will describe some modifications to the basic
scheme to account for two potentially critical effects in TD DCS experiments: non-diffusive effects and the effect of the IRF (i.e. finite temporal resolution of the detector).

4.2 – How to simulate TD DCS with Monte Carlo

In this section I will show the results of Monte Carlo simulations for TD DCS. For us, the system of interest is a semi-infinite turbid medium, as it is shown in Figure 4.3. The medium can be either homogenous or heterogeneous (an important particular case is the layered structure). To model a realistic TD DCS experiment we have considered a cube of turbid material surrounded by air. The overall dimension of our system are much higher than any other scale length (in our case \( L = 10 \text{ cm} \)). It is important to notice that, even if the physical parameters (for instance absorption distribution) vary in a continuous way along space, in a computer it is possible to save them only in a discrete set of points. For this reason, the cube is divided in small voxels, in our case with 1 mm size\(^8\). We place a collimated source at the surface of the medium, and a point-like (single voxel) detector at a distance \( \rho = 1 \text{ cm} \) from the source.

![Figure 4-3: Simulated system. In the left part of the figure, is possible to see the source and the detectors (red arrows) and the voxel domain. The geometrical and optical parameters are specified in the right part of the figure. For plotting reasons, the figure shows a voxel size of 1 cm, larger than the actual one of 1 mm.](image)

Geometric parameters:
- 10 * 10 * 10 cm cube
- 1 * 1 * 1 mm voxel
- \( \rho = 1 \text{ cm} \)

Optical parameters:
- \( \mu_s' = 5 \text{ cm}^{-1} \)
- \( \mu_a = 0.1 \text{ cm}^{-1} \)

We have chosen to investigate a medium composed by stacking two media over \( z \), as it shown in the picture. We have set the thickness of the top layer to \( t_{UP} = 1 \text{ cm} \), while the thickness of the bottom layer was set to \( t_{BOTTOM} = 9 \text{ cm} \), large enough to mimich a semi-infinite medium. Regarding the flow properties, we have chosen \( D_{B,UP} = 1 \times 10^8 \text{ cm}^2/\text{s} \) for the upper layer, and \( D_{B,BOTTOM} = 5 \times 10^8 \text{ cm}^2/\text{s} \) for the bottom layer (bilayer medium). As a control, we have also simulated a homogeneous medium with \( D_{B,UP} = D_{B,BOTTOM} = 1 \times 10^8 \text{ cm}^2/\text{s} \). The parameters are specified in Table 4.1.

\(^8\) The voxel size should be small compared to the scale length of variation of the physical parameters such as absorption distribution (in order to not loose information), but not too small in order to limit the memory usage. As a rule of thumb, the voxel size can be chosen to be \( \sim 1/\mu_s' \).
Table 4-1: dynamic parameters for the two simulated systems

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bilayer</th>
<th>Homogeneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_B$ [$cm^2/s$]</td>
<td>$1 \times 10^8$</td>
<td>$1 \times 10^8$</td>
</tr>
<tr>
<td>Upper layer</td>
<td>$1 \times 10^8$</td>
<td>$1 \times 10^8$</td>
</tr>
<tr>
<td>Bottom layer</td>
<td>$5 \times 10^8$</td>
<td>$1 \times 10^8$</td>
</tr>
</tbody>
</table>

Among of the first information that we can retrieve from the simulation is the absorption distribution of light inside the medium, see Figure 4.4. The absorption distribution is defined as the sum of the photon weights that have been dropped in each voxel, due to absorption. This information is contained in the Absorption file.

We used this Absorption distribution maps only as a control for our simulations, since in DCS (and in TD DCS) this information is not used.

For our purposes, the most useful information can be retrieved using the history file, see Figure 4.5. The history file is a big matrix that contains, for every photon packet detected, the path length in each of the medium composing the system, and the arrival weight of the packets. From the history file, knowing the speed of light in each medium, we can pass from the path lengths to the TOF using:

$$TOF = \frac{s_1}{v_1} + \frac{s_2}{v_2}$$  \hspace{1cm} (4-3)

Where $v_1 = c/n_1$ and $v_2 = c/n_2$ are the speeds of light in the two media (in general different, if the two layers have different refractive index).
Figure 4-5: Scheme of the history file: the rows correspond to the detected photon packets, the columns to paths in the medium and arrival weights.

With the TOF information and the weights it is possible to compute the DTOF in the following way:

$$DTOF(t_i) = \sum_{j=1}^{N_i} W_j$$  \hspace{1cm} (4-4)

where $t_i$ is central time of the $i$-th time bin, $W_j$ are the weights of the photons with TOF belonging to the bin, and $N_i$ is the number of photons detected in the $i$-th bin. In fact, the arrival weights can be interpreted as the probability of detection of the packets. It is important to notice that in a Monte Carlo (MC) simulation, all the photons are emitted by the source at the same time, not as in a real experiment in which the input pulse has always a certain temporal spread, and the temporal resolution of the detector is always finite.

Another important quantity that we can compute is the mean fraction of photon path length within each medium $P_{UP}(t)$, $P_{DOWN}(t)$. This quantity is defined as the weighted ratio of the photon path lengths in the considered medium divided by the total path length, for photons with TOF within a certain time bin $t_i$:

$$P_{UP}(t_i) = \frac{\sum_{j=1}^{N_i} s_{UP,j} W_j}{\sum_{j=1}^{N_i} W_j} \hspace{1cm} (4-5)$$

Of course, this quantity depends on time, since later photons typically travel deeper in the medium, and so we expect that the path length fraction in the bottom layer will increase by considering later gates (Martelli F., 2016). It is possible to show that this fraction is independent on the source detector separation $\rho$ chosen. However, the fraction of the photons that arrive at a late gate with respect to the total is smaller for smaller source detector separation. Figure 4.6 shows the computed DTOF and the fraction of path lengths $P_{UP}(t)$ and $P_{BOTTOM}(t)$ in upper and bottom layer.
As we can see from Figure 4.6, the simulated DTOF agrees well with the solution of the Diffusion Equation (and in particular with the extrapolated boundaries condition formula). For building the gated autocorrelation functions, one before builds the single path autocorrelation function for every detected photon (Zhou, 2007) as follows:

$$g_{1s}(\tau, s) = \exp(-2\mu_s^0[k_{0,UP}^2 D_{B,UP}s_{UP} + k_{0,BOT}^2 D_{B,BOT}s_{BOT}]\tau)$$  \hspace{1cm} (4-6)

Where UP and BOT subscripts refer to the upper and bottom layer\(^9\). After that, it is possible to compute the overall gated autocorrelation function simply summing the contributions of the single path autocorrelations, weighted on the path probability, and by considering only the packets within the gate \([s_1, s_2]\):

$$g_{1,[s_1,s_2]}(\tau) = \int_{s_1}^{s_2} P(s) g_{1s}(\tau, s) ds \rightarrow \frac{1}{\sum W_i} \sum W_i g_{1s}(\tau, s)$$ \hspace{1cm} (4-7)

Where we have estimated the path probability with the arrival weights and we consider in the summation only the packets with TOF belonging to the gate.

We have applied this method in the cases of bilayer medium (with different \(D_B\) in the two layers) and of homogeneous medium (with same \(D_B\) in the two layers), see Table 4.1. First, we

\(^9\) This expression holds in the limit of high number of scattering events (diffusive case)
have considered the case of narrow gates, with a time width of 100 ps. Figure 4.7 shows the resulting narrow-gates autocorrelation functions for the two systems.

![Autocorrelation Functions](image)

*Figure 4.7: Computed autocorrelation functions with narrow gates with 100 ps width. Earlier gates show a slower decay with respect to later gates, in both systems. The early gates show a similar behaviour for the two systems, while the later gates in the bilayer medium decay slightly faster with respect to the homogeneous medium, due to the larger deep flow.*

Then we have fitted the autocorrelation with a single-exponential model, since the narrow gating selects only a small interval of path lengths, and thus as a first approximation, the exponential can be taken out from the integral:

\[
g_1(\tau) \approx \exp(-k_s \tau) = \exp(-2\mu_s' D_B k_0^2 \tau)
\]  \hspace{1cm} (4-8)

Where we have defined \( k = 2\mu_s' D_B k_0^2 \), the rate of decay of the autocorrelation per unit of path length. Then, it is possible to make the substitution \( s = v(t - t_0) \), where \( t_0 \) is the starting time of the pulse and \( v \) is the speed of light in the medium\(^{10}\). The Figure 4.8 shows the fitted \( k_s \) as a function of time for the two systems: bilayer and homogeneous.

---

\(^{10}\) This relation is valid only if the refractive index is uniform into the system.
We have fitted the $k_s$ points with a line, considering separately early and late gates (the limit between early and late was set to 500 ps). Regarding to the late photons, only the gates for which $DTOF_{max}(DTOF) > 10^{-3}$ were considered for the linear fit. We can see from Figure 4.8 that, while for early photons the slopes of the $k_s$ points are the same, for late photons the slopes becomes significantly larger for the bilayer system. This is confirmed by the fitted values of $D_{B, late}$ that were $1 \times 10^{-8} cm^2/s$ for the homogeneous medium and $3.5 \times 10^{-8} cm^2/s$ for the bilayer.

We can see that the computed value of $D_B$ in the bilayer case is slightly smaller with respect to the simulated value (which is $5 \times 10^{-8} cm^2/s$). This phenomenon is probably due to the fact that, even if we consider only late photons, the fraction of path length in the bottom layer is always smaller than 1, see Figure 4.7. In any case, we can see that the narrow gates autocorrelations carry important information about localized changes in the flow.

Then, we applied the same method for computing broad gates autocorrelation functions from the simulation. Also in this case, the two systems (homogeneous and bilayer) has been considered. We have done three different gates:

- Early: from 0 to 90 % DTOF falling edge
- Late: from 90 % DTOF to 3 ns
- Ungated: from 0 to 3 ns

Figure 4.9 shows the resulting autocorrelation functions.
Figure 4-9: computed autocorrelation functions (early, ungated and late) for the two systems (homogeneous and bilayer). We set the limit between early and late photons to 90 % DTOF falling edge. Red, black and blue lines correspond to early, ungated (CW) and late gates. Continuous and dashed lines correspond to homogeneous and bilayer system.

From Figure 4.9 is it possible to see that, similarly to the narrow gates, later gates have a faster decay to zero. This because later gates have longer path lengths and the decay rate is proportional to the path length. It is essential to notice that broad gates autocorrelation cannot be described analytically neither as a single exponential (since the gate is not sufficiently narrow) nor as CW autocorrelation function, solution of the CDE (since we are not considering the whole span of path lengths). For this reason, for data fitting is necessary to consider the integral expression of the autocorrelation function. This will bring to accurate estimation of the value of the blood flow. From Figure 4.9, one notes also that the early gates functions overlap, while the ungated and the late gates have a different behaviour in the homogenous and in the bilayer system. This is expected, since the difference between the two systems is the bottom layer flow. We can also notice that the late gate changes more with respect to the ungated, and this confirms that the late gate have a higher deep flow sensitivity with respect to the ungated case. Furthermore, we can see that the change in the ungated and late autocorrelation is mainly in the smaller $\tau$ region. In fact, small $\tau$ part is determined by the photons that de-correlate faster, which are the photons that actually travelled more (longer paths). We will exploit this effect later in this Chapter.

In the following section, I will describe how I modified the MBio code in order to account for non-diffusive effects in the simulated autocorrelation functions.

4.3 - Non-diffusive effects

In the recent literature (Sutin K., 2016) it has been found that the rate of decay $k_s$ of the narrow gates autocorrelation functions has a slightly non-linear behaviour for very small TOF. For this reason, we have tried to determine with the help of simulations what could be the reason of this effect\textsuperscript{11}. Two effects could enter into play: non-diffusive effects and IRF effect. In this section, I am going to discuss the first, in the next I will discuss the second.

\textsuperscript{11} This non-ideality could bring to wrong estimation of the Blood Flow Index, thus is needed to be studied in detail.
Non-diffusive effects could distort the autocorrelation function because the decay-rate of the single path autocorrelation function is proportional to the number of scattering events, and for small path lengths, the number of scattering events can become non-linearly related to the path length. In fact, the single path autocorrelation function, in general, can be expressed as (Zhou, 2007):

\[ g_{1s}(\tau, Y) = \exp(-2k_0^2YD_B\tau) \]  

(4-9)

Where \( Y = \sum_i \left(1 - \cos(\theta_i)\right) \) is the sum of the a-dimensional momentum transfers that occur in the scattering events along the path. In the diffusive regime, it is possible to show that \( Y \simeq \mu' s \), and thus the single path autocorrelation is well described by the usual expression. Thus, to make the simulation in this scenario, it is necessary to know the \( Y \) value for every detected photon. For this reason, I have modified the MBio code to store the \( Y \) value in the history file, the same file in which the path lengths and the weights are stored. The history file, after this modification of the code, has the structure shown in Figure 4.10. In addition, a slightly modified version of the reader function for the history file has been developed, to be used when also the \( Y \) value is stored. In the MBio code, it is possible to select these new features by selecting the statistic of the correlator \( f_{corr} \) equal to 2 (non-diffusive case) in the input file.

![Figure 4-10: history file structure after the modification of the code. The Y values in each medium are stored together with the path lengths in each medium and the arrival weight.](image)

We then proceeded to the simulation of a semi-infinite medium with \( \mu'_s = 5 \text{ cm}^{-1}, \mu_a = 0.1 \text{ cm}^{-1}, \rho = 1 \text{ cm} \). We have set \( D_B = 1 \times 10^{-8} \text{ cm}^2/\text{s} \), uniform in the medium, and launched 10 Millions of photons.

**Narrow gates**

First, we have evaluated non-diffusive effects in the narrow gates case, using 10 ps gates (time short enough to study with sufficient detail the expected non-diffusive to diffusive transition). The single-path autocorrelation function has been computed using the expression (4.5). After that, the photons have been gated according to their TOF as in the diffusive case. The resulting gated autocorrelation functions have been fitted with a single-exponential model to
recover their decay rate $k_s$ as a function of time. Figure 4.11 shows the resulting decay rates $k_s$, together with the relative difference between the decay rates $k_s$ in the diffusive and non-diffusive case.

From Figure 4.11 is it possible to show that in the diffusive case the $k_s$ points are disposed along a line, while in the diffusive case there is a certain variation with respect to the linear trend. However, the difference between the two is present only for very short times. For times longer than 80 % DTOF rising edge, the difference starts to decrease towards zero. For this reason, as a rule of thumb, we have always neglected the gates before that time in the data analysis, to recover an accurate value of the $D_B$. To understand better the reason of this deviation from the linear trend, in Figure 4.12 I plotted the average number of random walk steps $\mu_s$ against the adimensional momentum transfer $Y$, as a function of time.

From Figure 4.12 it is possible to notice that the behaviour of $Y$ is not well approximated by its ensemble average ($\mu_s$) for small times. Since the decay rate of the autocorrelation function is proportional to $Y$, this can explain the distortion of the $k_s$ versus $t$ plot from the linear trend (see Figure 4.11). We have to note that the effect is noticable only for times smaller than $100 - 200\, ps$, for larger times it does not introduce significant effects on the autocorrelation decay rates. Thus we can conclude that, in the narrow gates case, non-diffusive effects can be tackled simply by neglecting this very first part of the DTOF in the computation of the autocorrelations.

Figure 4-11: Non-diffusive effects, narrow gates. Upper plot: computed values of $k_s$, for the non-diffusive and diffusive case. Lower plot: relative difference in the decay constant $k_s$ between non-diffusive and diffusive case.
Figure 4.12: histogram of Y and of $\mu'_s$ as a function of time. For small times, the Y is not well approximated by $\mu'_s$. The effect is negligible for times larger than few hundreds of picoseconds.

**Broad gates**

As we will see in the next chapter, to obtain sufficient SNR in an *in vivo* experiment, it is preferable to use the *broad gates*, since in that case more photons are collected per unit time. In order to simulate this case, we have used the same method as before, i.e. computing the correlation in post-processing from the history file. In this case, we have applied three gates to the TOFs: early, late and ungated. The separation between early and late was set to 90% DTOF falling edge. We have computed the $g_1(\tau)$ in the general non-diffusive case and in the diffusive limit. A fit of the autocorrelation curves with the solution of the CDE showed no convergence to the simulated autocorrelation and values of $D_B$ significantly different from the expected ones.

In order to fit better the data in this large gates case, we have used the more accurate expression for $g_{1,\text{Theor}}(\tau)$, the theoretical model that we use for the fit:

$$g_{1,\text{Theor}}(\tau, D_B) = \int_{s_{\text{IN}}}^{s_{\text{FIN}}} P(s) g_{1,s}(\tau, s, D_B) ds$$  \hspace{1cm} (4-10)

Where $s_{\text{IN}}$ and $s_{\text{FIN}}$ denote the limiting path-lengths of the gate, and we have made explicit the dependance of $g_{1,\text{Theor}}(\tau)$ on the $D_B$.

Given an experimental or simulated $g_{1,\text{EXP}}(\tau)$, the new algorithm for the fit follows these steps:

1. Numerically solve the integral to get $g_{1,\text{Theor}}(\tau, D_B)$, using a trial value for $D_B$.
2. Compute $\chi^2(D_B) = \sum_i |g_{1,\text{Theor}}(\tau_i, D_B) - g_{1,\text{EXP}}(\tau_i)|^2$
3. Find $D_B^* = \arg\min_{D_B} \chi^2(D_B)$ using an iterating minimization procedure\(^{12}\)
4. Compute the fitted autocorrelation as $g_{1,\text{Theor}}(\tau, D_B^*)$

\(^{12}\) For example using the Matlab function fminsearch
Figure 4.13 shows the simulated autocorrelation curves and the fit with the numerical integration, for the diffusive and non-diffusive case.

![Autocorrelation curves](image)

From Figure 4.13 it is possible to see that, in the diffusive limit we have a good fitting of the autocorrelation curves with the new model, for all gates. On the other hand, the non-diffusive autocorrelations are fitted more difficulty by the model, especially the early gate. This is reasonable, because early photons do a shorter path in medium, and thus the ensemble average on the scattering angles can be less accurate to estimate the adimensional momentum transfer $Y$ with the path length. However, it is worth noting that the fit quality is lower for higher $\tau$, in regions in which the autocorrelation is lower than $\sim0.25$. This can be explained by the fact that non-diffusive effects are stronger for smaller path lengths, and the effect of small path lengths on the autocorrelation is mainly for large $\tau$. Figure 4.14 shows the retrieved values of $D_B$ with the use of the numerical integration fit, and the residual sum of squares $\chi^2$, for the autocorrelations shown in Figure 4.13.

Figure 4.14 shows that, even in the non-diffusive case, the retrieved values of $D_B$ are close to the expected one. However, the quality of the fit for the non-diffusive case is smaller than the diffusive case, and degrades passing from late to early gates. One method to tackle this problem could be to neglect in the fit the points for which the autocorrelation is smaller than a given threshold, i.e. consider only $g_1 \geq g_{\text{threshold}}$. This because the small $\tau$ region is due mainly to photons that have traveled more in the medium, and thus we expect the non-diffusive effect for those photons to be smaller. In this way the quality of the fit, and thus the reliability of the $D_B$ estimate, could increase in a significant way.

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Figure 4-14: Retrieved values of $D_B$ and residuals (i.e. sum of squares), for the broad gates autocorrelations of Figure 4.13. For the three gates considered (early, ungated and late), we compare diffusive and non-diffusive case (blue and yellow bars).

The use of Monte Carlo simulations gave us the opportunity to test a new fitting method for the gated autocorrelation function, which showed to give more accurate results with respect to the fitting method based on the solution of the CDE. An advancement in the MC code gave us the opportunity to gain physical insight on non-diffusive effect on the autocorrelation functions.

We have to note that, for now, in these simulations we have neglected effects arising from the finite duration of the input light pulse and from the finite detector temporal resolution (i.e. the effect of the IRF). In the next section, I am going to discuss how we modified the post-processing routine to include this effect on the computation of the autocorrelation functions. In addition to that, I will show how the use of a theoretical model that accounts for the IRF itself permits a data fitting that gives accurate values of the blood flow index.

4.4 – Effects of the IRF

In a TD NIRS experiment, since the response time of the detector is comparable with the temporal duration of the theoretical reflectance, the IRF brings to a temporal broadening of the detected curve, thus the measured curve (DTOF) is different than the theoretical reflectance. The effect of the IRF in a TD NIRS experiment is modelled with the convolution of the theoretical reflectance with the measured IRF. On the other hand, to our knowledge it has been never discussed in the literature how the IRF distort the autocorrelation curves in a TD DCS experiment. In Section 3.3 I have provided a model that attempts to include the IRF in the expression of the measured gated autocorrelations.

In this section I will discuss how I modified the post-processing algorithm to include the effect of the IRF in simulated autocorrelation curves. Then, I will describe the results of the simulations.
4.4.1 – Including the IRF in Monte Carlo simulations

In an ideal TD DCS experiment, the time-of-flight (TOF) of each photon is measured with perfect accuracy, enabling a precise estimation of the true path length $s'$ of each detected photon. Instead, in a real TD DCS experiment, the TOF is not estimated with perfect accuracy due to the finite temporal resolution of the detector (and the duration of the input pulse). For this reason, we estimate the path length of the photon to be $s_0$ (apparent path length) instead of $s'$ (real path length). When then we correlate the photons, since the rate of decay of the autocorrelation is due to real path length, but we assign the photons to the gate according to the apparent path length, we will have a distortion of the autocorrelation curve\(^{14}\).

To model this phenomenon, we can decompose the measured time of flight (TOF') as a sum of the real time of flight (TOF) and a certain temporal shift due to detector inaccuracies and pulse duration:

$$ TOF' = TOF + t_{\text{SHIFT}} $$  \hspace{1cm} (4-11)

Every detected photon will have a specific $t_{\text{SHIFT}}$ that will be a continuously distributed random variable. The distribution of $t_{\text{SHIFT}}$ is given by the IRF itself, because the IRF can be interpreted as the temporal spread at the output, for a delta of Dirac input pulse. Thus, the only thing that remains to do is to sample in a correct way $t_{\text{SHIFT}}$ using the IRF distribution. However, Monte Carlo already provides an easy method to sample random variables, which is called Basic Monte Carlo sampling and has been discussed in section 4.1.1. Thus, a possible algorithm to follow could be:

1. Assume a certain analytical expression for the IRF (Gaussian, step, etc.), or use an experimentally measured IRF
2. Compute (numerically) the Cumulative Distribution Function of the IRF, for a sufficiently fine set of temporal points
3. Use random sampling to compute a succession of $t_{\text{SHIFT}}$ values, one for every detected photon (see Section 4.1.1)
4. Compute TOF' according to equation (5-11)
5. Assign the photons to the gates according to TOF'.
6. Compute the single path autocorrelation functions with decay given by the true path length
7. Compute the final autocorrelation function using step 5 and 6.

As already discussed in section 3.3, the main theoretical difficulty to model this effect is that while the decay rate of the autocorrelation is due to the real path length, the photons are correlated according to their apparent path length. In fact, the decay rate depends on the number of scattering events that is independent on the detector resolution (we are assuming that the resolution of the detector is much finer than the time scale at which the autocorrelation starts to decay to zero).

\(^{14}\) In this discussion, we are assuming a uniform refractive index $n$ in the medium, in order to be able to pass from the path length to the time of flight simply by using the (uniform) medium’s speed of light $v = c/n$. 

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4.4.2 – IRF effect on simulated gated autocorrelations

In this section, I am going to present the results of the simulations regarding the effect of the IRF on gated autocorrelation functions. I have considered the same system of the previous sections of this chapter, i.e. a semi-infinite turbid medium with $\mu'_s = 5 \text{ cm}^{-1}$, $\mu_a = 0.1 \text{ cm}^{-1}$ and a SD separation $\rho = 1 \text{ cm}$. For now, I consider the system to be homogeneous, later in the section I will consider a bilayer medium.

Narrow gates

I have first considered the case of narrow gates autocorrelation functions, with a time width of 100 ps without overlap. To compute the autocorrelations, I have used the method described in section 4.4.1, in which I considered as an IRF a Gaussian function 400 ps width. Then as a comparison I have also computed the autocorrelations in the case without IRF. The $D_B$ was set to $1 \times 10^{-8} \text{ cm}^2/s$ in all the cases. Figure 4.17 shows the resulting autocorrelation functions.

![Autocorrelation functions](image)

*Figure 4-15: Narrow gates (100 ps) autocorrelation functions for an ideal system and for a system with a 400 ps FWHM Gaussian IRF. Colour map goes from blue for the first autocorrelation to red for the last autocorrelation. Note that the plot scale is $(x, y) = (\text{lin}, \text{log})$.*

After the computation of the autocorrelation function, I have fitted them with a single exponential to recover the decay rate $k_s$ as a function of $t$. Figure 4.17 shows the resulting decay rates $k_s$, for four different cases: no IRF, 100 ps, 200 ps and 400 ps IRF (FWHM).
Figure 4.17 shows that, without IRF, the narrow gates autocorrelations are approximately single exponentials, as foreseen by the theory. In fact, the functions are lines in a $\text{lin} - \text{log}$ plane. When instead we add the effect of the IRF, the autocorrelations get curves, and this means that the function is not anymore describes by a single exponential. This happens because the IRF maps photons with neighbouring path length into the gate, thus we have a certain distribution of path lengths within the gate, even if the gate is very narrow. Note that this effect is not readily explicable by inspection of the DTOF.

Regarding the decay rate, we see that it does not anymore follow a linear trend if plotted against time. The deviation of the decay rate, when we introduce the IRF, is bigger for smaller path lengths and increases with increasing width of the IRF. This can be explained by the fact that the decay rate is proportional to the path length, thus for small path lengths the confounding effect of the IRF will give a bigger relative uncertainty in the decay rate itself. Additionally, we can see that the effect of the IRF on the decay rate is present for TOF of the order of width of the IRF itself, thus for a 400 ps IRF the effect is present up to roughly 1.5 ns. Later in this chapter, we will see how is it possible to solve this problem using the theory developed in Section 3.3, and by avoiding computing the decay rate by directly calculating the BFI.
Broad gates

After having considered the narrow gates case, I have applied the same method to compute the effect of the IRF on the broad gates autocorrelation functions. I have considered the same system of before, and applied three temporal gates, early ungated and late. The separation time between early and late was set to 90% DTOF falling edge as usual. This time I have considered more values of IRF FWHM, choosing values from no IRF to 1000 ps, see Figure 4.17. Note that the IRF effect is added in post-processing, thus it was possible to launch a single MC simulation and then add the IRF.

It is important to note that, while in an ideal experiment the photons arrive always for positive times, adding a symmetric IRF introduces the possibility for a photon to arrive at a negative time. This effect, especially for early and ungated cases, makes us not correlate photons that should be into the gate (the photons go to negative times and they exit from the gate). For this reason, we have anticipated the opening of the gate by 1 ns for the early and ungated cases, to not loose those photons. This method clearly is not useful for the late gate since the early photons can both enter and exit from the late gate.

![homogeneous medium autocorrelation functions for different gaussian IRF](image)

*Figure 4-17: Broad gates autocorrelation function for a homogenous medium. The colour scale, from blue to red, corresponds to increasing values of IRF FWHM from zero (no IRF) to 1000 ps.*

We can see from Figure 4.17 that the ungated case is not affected, since with the anticipation of the opening of the gate we are collecting the whole DTOF, thus correlating the right photons. On the other hand, early and late gates are both affected by the IRF. The early gate is much more affected than the late gate, and probably this is because the relative uncertainty in path length (and thus in decay rate) for the early photons is higher than the late photons.

\[^{15}\text{We define time zero the time at which the IRF has its peak.}\]
In addition to that, we can see that while the late gate is affected only in the large $\tau$ region, the early gate is affected both for small and large $\tau$ in a similar way.

As we can see that, in the late gate, the small $\tau$ region of the autocorrelation is not changing a lot if we add the IRF, a simple way to reject its effect is consider in the fit only that part of the curve. More specifically, one considers in the fit only points such that $g_1 \geq \gamma_{\text{threshold}}$, with $\gamma_{\text{threshold}} \sim 0.7$. In fact, we can see from Figure 5-19 that for points above a horizontal line with height 0.7 the autocorrelation with and without IRF are not very different from each other. This method can be called “double gating”, because the region with small $\tau$ correspond mainly to photons that undergo bigger path lengths, thus we are even more sensitive to the longer path photons with this method. On the other hand, for early gate this strategy is not useful since the autocorrelation is affected both for large and small values of $\tau$. Figure 4.18 shows the estimated values of the BFI (normalized to the expected one), obtained with the strategies described in this part.

![Figure 4.18](image.png)

*Figure 4.18: Estimated value of the $D_B$, normalized to its expected value. The double gating method (see text) have been applied to the late gate, and as a comparison it is shown also the result without double gating method. In the early and ungated (CW) cases, the anticipation of the opening of the gate (see text) have been applied.*

We can see from Figure 4.18 that the methods developed so far are not completely effective for rejecting the effect of the IRF. In the case of ungated acquisition, the BFI is close to the expected one. In the other two cases, the strategies of anticipation of the opening of the gate and of the double gating improve a little bit the situation but, especially in the early gate it is necessary to find a more effective method for fitting the data.

From these results we can see that, both in the narrow and broad gates, the fitting strategy developed so far does not permit us to find accurate values of the BFI. For this reason, we have developed a theory that attempts to include the IRF in the expression of the measured (or simulated) gated autocorrelation function. This theory has been discussed in section 3.3. In the remaining part of this section, I will describe how I applied the theory for developing a new fitting algorithm that includes the effect of the IRF. As we will see, this new algorithm permits to recover more accurate values of the BFI from gated autocorrelations, both in the narrow and in the broad gates cases.
4.4.3 – Fitting the simulated autocorrelation with the IRF-corrected model

In this section I will discuss how the estimation of the BFI changes when, in the fit of the gated autocorrelations, we use the model that considers the effect of the IRF (see Section 3.3).

**Broad gates**

We consider a system with the same optical properties of the previous Section, i.e. semi-infinite with $\mu'_s = 5 \text{ cm}^{-1}, \mu_a = 0.1 \text{ cm}^{-1}$ and a SD separation $\rho = 1 \text{ cm}$. Now I will consider the case of broad gates, later the case of narrow gates. To model the effect of the IRF we need to plug into the integral expression of the autocorrelation the $\theta(s')$ function that, in the case of broad gates, can be seen as the cross-correlation between the IRF and the gate itself (see Section 3.3). Figure 4.19 (a) shows the IRFs that I have considered, while Figure 4.19 (b-c-d) shows the computed $\theta(s')$ functions for early, ungated and late cases.

![Figure 4.19: Upper left plot: considered IRFs (coloured lines) and simulated reflectance (dashed line), in logarithmic scale. Upper right and lower plots: computed theta functions (coloured lines) and simulated reflectance (dashed line) for the three cases of early, ungated and late, in linear scale. In all plots the colour map (from blue to red) correspond to increasing width of the IRF (from zero to 400 ps). The separation between early and late gate has been set to 0.5 ns.](image)

The theta function can be seen as the probability that a photon with true path length $s'$ is assigned to the gate $[a, b]$. When we don’t have the IRF, this probability is equal to one for all the path length within the gate. On the other hand, when we add the IRF, this probability gets distorted, in particular close to the edges of the gate. In the case of early gate, a photon that has a path length close to the end of the gate has a probability $< 1$ to be assigned to the gate, since it could be mapped by the IRF in a region after the gate itself. In the ungated case we have the same effect, but since the end of the gate happens when the $P(s')$ is very small, this does not introduce a significant effect in the autocorrelation. In the case of the late gate it happens the opposite: there is a certain probability that a photon that comes before the
opening of the gate is assigned to it. In the late gate, the effect at the closing of the gate is negligible because the $P(s')$ is very small (as in the ungated case). Thus, from the behaviour of the theta functions, for the early gate we can see that we are collecting less photons that have lower path length than expected, thus the autocorrelation will decay faster, and this is interpreted (by the uncorrected fit) as a larger BF. On the other hand, in the late gate we are collecting some photons with smaller path length, thus the autocorrelation will decay slower and this is interpreted as a smaller BF.

Figure 4.20 shows a comparison between the uncorrected and IRF corrected fits (i.e. considering the IRF in the model for data fitting) in the case of simulated broad gates autocorrelation functions. The fits are compared showing the residuals and the estimated BFI for the three cases of early gate, ungated acquisition and late gate. The fits have been performed from $\tau = 10^{-6}$ s to the lag time such that $g_1 = 0.5$.

![Image of Figure 4.20](image)

*Figure 4-20: Left plots: Simulated broad gates autocorrelation functions (blue circles), uncorrected and IRF corrected fits (red and black lines). Central plots: residuals for the uncorrected and IRF corrected fits (red and black lines). Right plots: Comparison between the expected BFI (no IRF) and the BFI retrieved with and without IRF correction (irf 400 ps). The fits have been performed from $\tau = 1E^{-6}$ to the lag time such that $g_1 = 0.5$.*

We can see from Figure 4.20 that, considering the IRF in the fitting of the simulated data, we obtain a higher fit quality for all the values of lag times. Regarding the estimated BFI, we can see that correcting for the IRF we obtain a BFI very close to the expected one, also in the case of early and late gates.

**Narrow gates**

In a similar way with respect to the broad gates, I have checked whether we can correct the effect of the IRF on the narrow gates autocorrelation functions, by using the model discussed
in Section 3.3. I have considered the same system as before, but this time I considered 100 ps long gates (non-overlapped).

When we do not have the IRF, in a narrow gate we have a very narrow distribution of path lengths (in the limiting case a delta of Dirac centred at the middle of the gate $<s> = \frac{a+b}{2}$). When instead we add the IRF, we will have a certain distribution of path lengths within the gate, and this does not permit us to define a decay rate $k_s$ to the autocorrelation function (because the autocorrelation is not anymore a single exponential). For this reason we found that it is more accurate to compute directly the various $D_B$ from each autocorrelation function (by using the model in Section 3.3), instead of computing all the $k_s$ and then make a linear fit to recover a single $D_B$, as in (Sutin K., 2016). Thus, the difference with respect to before is that now we get directly a series of $D_B$ values, one for each gate. Figure 4.21 shows a comparison between the two fitting methods (uncorrected and IRF corrected) for the case of simulated homogeneous medium and a 400 ps width Gaussian IRF.

![Figure 4-21: homogeneous system narrow gate autocorrelation functions. Gate width 100 ps without overlap, Gaussian IRF with width 400 ps FWHM. Upper plot: Two indicative autocorrelation functions, corresponding to a gate centre at 50 ps and at 550 ps (1st and 5th gate, red and blue). The diamonds, dashed lines and continuous lines correspond to simulated, uncorrected fit and IRF-corrected fit. Lower plot: comparison between the DB retrieved with the uncorrected and IRF-corrected fit (continuous and dashed black lines). The vertical dotted lines (red and blue) enclose the gate regions of the two autocorrelation functions shown in the upper plot.](image)

From Figure 4.23 we can see that the quality of the fit with the IRF-corrected method improves significantly, especially in the large lag time region. Note how the IRF corrected fit follows correctly the deviation of the autocorrelation functions from the single exponential trend (that corresponds to a line in the logarithmic plot). When we compute the BFI for each gate
(as described before) and we use the new model, we see that we recover a fairly constant value of the $D_B$, very similar to the expected one.

In this Section, I have shown that the use of the proposed model for data fitting permitted us to recover more accurate values of the BFI, for both narrow and broad gates. The use of Monte Carlo simulations gave us useful physical insight on the dependence of the TD DCS signals on several effects, and permitted us to test new fitting algorithms that can be useful also for real experiments data analysis.
Chapter 5 – Experimental setup and correlation methods

In this Chapter, I am going to present the setup used in the experiments. After that, I will discuss briefly the Software correlation method employed to compute the intensity autocorrelation functions starting from the stream of photons.

5.1 – Experimental setup

The TD DCS experimental setup can be divided conceptually in three parts: pulsed light source, detection of the diffused light, and light intensity autocorrelation computation (M. Pagliazzi, 2017). Figure 5.1 shows a schematic diagram of the setup.

![Figure 5-1: Experimental setup diagram. The red lines identify laser light in free propagation. The black arrows identify electrical signals. MM and SM stand for Multi-Mode and Single Mode fiber. For space reasons, the pump laser (Nd:Yag) for the Ti:Sapphire laser is not draw.](image)

In the experiments, we used a Ti:Sapphire mode locking laser. A mode locked laser permits the generation of a train of identical light pulses with duration ~ $ps - ns$, and with temporal separation equal to the time that light takes to make a round trip in the cavity. In our source, the light pulses are obtained using an active mode locking method, with the use of an acousto-optic modulator. Many other mode locking techniques exist, for instance passive mode locking. The acousto-optic modulator permits to periodically modulate the cavity losses. If the frequency of modulation is equal to the difference in frequency between two successive longitudinal modes, we can obtain a phase locking of the modes, i.e. the modes of the cavity have a well-defined phase relation (Svelto, 2010). This permits to obtain a light source with high temporal coherence.

The Ti:Sapphire laser is pumped using a Continuous Wave Nd:Yag laser, which was frequency doubled and has a 5 $W$ optical power. The laser repetition rate, fixed by the cavity length, is 100 $MHz$, while the lasing wavelength can be tuned in the 680 – 1090 $nm$ range.
application, the wavelength was set to $\lambda = 785\ nm$. After the source, light was split in two parts. ~5 % of the light was sent to a photodiode for synchronization signal generation. The remaining part was optically attenuated and sent to the sample. The optical attenuation is achieved with a series of three metallic variable attenuators, and in an \textit{in-vivo} experiment is necessary for keeping the light mean and peak intensity below the skin Maximum Permissible Exposure (MPE) limits. After the attenuation, light was coupled to a 200 $\mu m$ core diameter multi-mode fiber and delivered to the surface of the sample.

After the light is diffused in the sample, it is collected with a single mode fiber, with core diameter 5 $\mu m$. In detection, the single mode fiber is used to detect a single speckle, and thus to maximize the value of the $\beta$ parameter. The Source Detector (SD) separation was $\sim1\ cm$. The precise value of the SD separation is chosen according to the specific experiment requirements. After the fiber, light is coupled to a Single Photon Avalanche Diode (SPAD) detector. We have chosen a detector with a sufficient photon detection efficiency, in our case $\approx20\ %$ for our wavelength, and a high temporal resolution (see Section 2.3). The used SPAD detector has two outputs: a Nuclear Instrumentation Module (NIM) with a fine ($\sim35\ ps$) temporal resolution, and a Transistor- Transistor Logic (TTL) with a coarser ($\sim250\ ps$) resolution.

For the computation of the gated autocorrelation functions, the detector’s NIM output and the sync signal were connected to a commercial TCSPC module. For every detected photon, the TCSPC module saves in a file the delay of the photon detection with respect to the sync signal, with a 8 $ps$ temporal resolution and the absolute arrival time of the photon, expressed as a multiple of the pulse’s repetition period (in our case 10 $ns$). This method of acquisition is called time-tagged time resolved (TTTR) acquisition method.

Since the pulse repetition rate was higher than the maximum admitted by our TCSPC module (84 $MHz$), we have decided to select one sync signal out of three with the use of a custom-made circuit. Consequently, the TCSPC histogram contains three different DTOF curves, arising from three successive pulse repetitions. These three curves were then combined in data analysis to not loss counts\textsuperscript{16}. The TCSPC file then is fed into a computer to compute, with a specific software, the intensity autocorrelation function. I will discuss the principle of this software in Section 5.2.

The other output of the SPAD (the TTL) is directly connected to a hardware correlator. This device computes the intensity autocorrelation function assuming that the light is continuous wave, i.e. it does not apply any temporal gate. Thus, in the present setup, the hardware correlator can be used only for the computation of the ungated autocorrelation functions, and was used for comparison and validation of the software correlator.

In some cases, as a light source we have used a continuous wave high coherence diode laser (Crystal Laser), as a comparison with the Ti:Sapphire source. This permits to compare the values of the BFI retrieved with the TD DCS technique and with the standard DCS technique.

\textsuperscript{16} Since the pulse separation (10 $ns$) is much smaller than the time scales at which the intensity fluctuates ($\sim\mu s - ms$), we can assume that this operation does not distort the autocorrelation functions.
Before the actual measurement, the IRF needs to be measured. To do that, the input and output fibers are placed one in front of the other, a thin film is placed between the two, and the resulting TCSPC histogram is measured. The thin film is used for intensity attenuation (to not saturate the detection electronics) and for mode mixing. The typical duration of the IRF spans from 100 ps to 400 ps and, for a given detector, depends mainly on the laser settings (like the voltage applied to the acousto-optic modulator).

5.2 – Software correlation method

As it is illustrated in Figure 5.1, our experimental setup is compatible with two different correlation methods: software and hardware correlation. Hardware correlators are commercially available devices that compute, in real time, the autocorrelation function of a photon stream. Some of them can also record the intensity profile of the detected light, on time scales. Regarding to TD DCS, their main disadvantage of commercial hardware correlators is that they do not have a way to path-length gate the photons, unless if coupled with a fast-gated detector (gating method that can be called “hardware gating”). Another disadvantage is that since the correlation is computed in real time, it is not possible to discard in post-processing unwanted parts of the data. On the other hand, it is possible to use a commercial TCPSC device (see setup in Section 5.1) and suitable correlation algorithms to compute gated autocorrelation functions in software. Software correlation is based on the TTTR data acquisition method discussed in Section 5.1, where the delay with respect to the sync pulse and the absolute (macroscopic) arrival time are recorded, only when a photon is detected. In this case, the data flow is directly proportional to the photon detection count rate (Michael Wahl, 2003). The problem of conversion of TTTR data in a correlation curve has been first faced in the context of Fluorescence Correlation Spectroscopy (FCS).

I used a software correlation algorithm developed by some colleagues at ICFO, and here I will present the basic principles of it. First, I will consider the computation of ungated autocorrelation function (starting only from the photons arrival time), and then I will discuss how it is possible to generalize it to the gated case (using the photons time of flights). A simple approach for computing the ungated autocorrelation would be to evaluate, with uniform time binning, the detected intensity starting from the photons (macroscopic) arrival times and to compute from that the autocorrelation function, but this would generate enormous files during the computation. A feature of TTTR acquisition is that the photons arrival times are all multiples of some minimal time $\delta t$, in our case the repetition period of the laser $\sim 10 \text{ ns}$. The photons arrival times can then be sorted in a linear array of time tags $\{t_1, t_2, ..., t_N\}$ where $N$ is the total number of detected photons. For simplicity these times can be measured in units of $\delta t$. The autocorrelation function definition is:

$$g_2(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)^2\rangle} \quad (5-1)$$

Where the brackets denote average over $t$. Since it is only possible to detect one photon in each time bin, the intensity can be either 0 or $1/\delta t$ depending if the photon is detected or not. Then the temporal average $< \cdots >$ can be computed summing over all the possible time bins, divided by the number of summed intervals.
The key point that permits to reduce the computational time is that the values of the lag times $\tau$ are not considered to be linearly spaced, instead one gradually increases their spacing. Typically, the lag times are chosen to be approximately logarithmically spaced. This choice of time lag spacing was first introduced in hardware correlators. A typical choice of the lag times is the following:

$$\tau_j = \begin{cases} 
1 & \text{for } j = 1 \\
\tau_{j-1} + 2^{\text{int}(\frac{j-1}{B})} & \text{for } j > 1 
\end{cases} \quad (5.2)$$

Where $j$ is an integer that goes from one to some maximum value $j_{\text{max}} = n_{\text{casc}}B$, where $B$ is some integer base number and $\text{int}(\ldots)$ gives the integer part of the number enclosed in the brackets. Eq. (5.2) generates $n_{\text{casc}}$ groups of lag times. Each group, so-called cascade, is composed by $B$ time lags with equal spacing $2^{\text{int}(\frac{j}{B})}$. This choice of the lag times is advantageous because all the lag times $\tau_j$ are integers numbers so the computation is faster.

The algorithm works directly on the arrival times $t_i$, instead of converting them in time-binned data. First, a value of the lag time $\tau$ is fixed, and from that a second vector of arrival times $t'_i = t_i + \tau$ is generated. At the beginning, $g_2(\tau)$ is set to zero. Then, it looks for correspondences between the $t$ vector and the $t'$ vector, and every time that $t_i = t'_k$ (for some integers $i$ and $k$) it adds one to the autocorrelation function. This method basically computes the probability that a photon is detected at time $t + \tau$, when a photon at time $t$ was detected. The autocorrelation computed in this way misses some important features of the detected intensity, for instance in the case of periodic signals with period not included in the vector of lag times $\tau$. For facing this, for increasing lag times it is applied an averaging procedure on the photon arrival times, to coarsen their temporal resolution. This method is equivalent to the multi-tau scheme used in hardware correlators. In the algorithm, it is implemented by associating to each value of $t_i$ and $t'_i$ some weights $w_i$ and $w'_i$. These weights are all set to one at the beginning. When one passes from one cascade of $B$ lag times to the next cascade. In case of equality of the arrival times $t_i = t'_k$, the autocorrelation is increased by $w_i w'_k$ and not by one. When one passes to the computation of the autocorrelation for a lag time belonging to the successive cascade, all time arrival times $t_i$ are divided by two and only their integer part is considered. When in the new vectors of arrival times $t_i$ there are two equal numbers, just one of the two is kept and its weight is increased by the weight of the eliminated one. To correct for the increased time scale, one divides the autocorrelation function by the factor $2^{\text{int}(\frac{j}{B})}$. The algorithm coarsens the time resolution as bigger lag times are considered, and this decreases more and more the number of arrival times to be considered in the computation when one arrives to the last lag times.

**Time-of-flight gated (and macro-time gated) autocorrelation functions**

For now, we have considered the computation of ungated autocorrelation functions, i.e. all the time of flights are considered in the computation. The extension to the gated case in conceptually simple. One needs to consider only the photons with time of flight TOF belonging to the considered temporal gate ($TOF \in [a, b]$, where $a$ and $b$ are the limits of the gate, for instance $1 \text{ ns}$ and $1.2 \text{ ns}$), and then compute the autocorrelation function with the algorithm described above (M. Pagliazzi, 2017). When multiple gates are considered one need to run one computation for each temporal gate.
It is important to note that, in certain applications, it can be interesting to consider in the computation only a specific time window of the experiment. This is done with the so called macro-time gating. The macro time gating is implemented in a similar way than the time-of-flight gate: only the photons belonging to a specific time of arrival gate \( t_i \in [A, B] \) (where \( A \) and \( B \) are the limits of the gate, for instance 100 s and 130 s) are considered in the computation. In this case, it is important to rescale the vector of the time of arrivals \( t_i \) to the initial time of the gate \( A \). In this way the process of time coarsening of the \( t_i \) works correctly.

In addition to this, the code at my disposal has the possibility to compute the DTOF of the detected light in a given time of arrival interval \([A, B]\) (to be used for TD NIRS analysis) and the detected light intensity profile with a specified temporal resolution (for instance 1 s), useful for instance for checking laser stability during the experiment.
Chapter 6 - Experimental results

In this Chapter I will present the experimental results regarding TD DCS measurements taken during my Thesis period. For the experiments, we have used the setup described in Section 5.1. When not specified, the autocorrelation functions have been calculated with a Phyton software correlator, that have been recently developed at ICFO. The principle of the algorithm has been described in Section 5.2. In some cases, as a comparison, we have also used a hardware correlator (correlator.com), that permits to compute and save the autocorrelations functions in real time, but without the possibility of time gating. For time reasons, the results shown in this Chapter has been obtained using the “standard” TD DCS models (i.e. without IRF correction).

The experiments that have been carried out can be divided into two categories: phantom and in-vivo studies. The first three sections of the Chapter will be devoted to the description of the phantom studies, the last ones to the in-vivo studies. Regarding to the phantom studies, we have carried out experiments on homogeneous and heterogeneous (bilayer) phantoms. On the other hand, in the in-vivo experiments we focused to the study of the brain and of the muscle of adult healthy subjects.

6.1 – Phantom experiment: changing the scattering coefficient

Our first TD DCS experiment was done on tissue-mimicking liquid phantoms (made of water and lipofundin). Three different phantoms have been done, in which we have changed the value of the reduced scattering coefficient $\mu'_s$ by changing the concentration of lipofundin. We considered three nominal values of $\mu'_s$: 5, 6 and 7 cm$^{-1}$. As already discussed in Section 5.1, due to constrains on the maximum sync frequency of the TCPSC module, the measured TCSPC histogram will be composed of three curves (each of one separated by 10 ns), corresponding to three successive repetitions of the pulsed laser source. Figure 6.1 shows the measured DTOF and IRF for the three phantoms considered.
Since the theoretical expression of the reflectance depends on $\mu'_s$, the measured DTOF should change for different values of $\mu'_s$. On the other hand, we can see from Figure 6.1 that the experimental DTOFs are very similar one to each other. To understand why this happens, we should check if the $\mu'_s$ of our phantoms have the correct values. To do that, one needs to do a TD NIRS fit of the DTOF (see Section 3.1). After that, we should verify that the fitted $\mu'_s$ are equal to the nominal ones ($5, 6, 7 \text{ cm}^{-1}$).

Considering the three curves in a separate way for the computation of the autocorrelation decreases the count rate and thus degrades the SNR. For this reason, the software correlator developed by other colleagues combines the three curves together before computing the autocorrelation (see Section 5.1), a procedure called “wrapping”. We have verified that the wrapping procedure does not distort the autocorrelation curves. The method was applied to compute the autocorrelations for 100 ps width gates, with 50 % overlap.

In addition to that, the software correlator at my disposal has the capability of calculating the intensity profile of the detected signal over time. It is important to check that the laser intensity is stable over time because, as I will discuss later in this Chapter, light source intensity fluctuations have a strong impact on the quality of the correlation curves.

In Figure 6.2 I show the narrow gates autocorrelation curves for the three phantoms considered.
We can see from Figure 6.2 that the measured autocorrelation functions show a high value of the $\beta$ parameter ($\sim 0.3 - 0.4$), close to the value expected with a CW laser source ($\beta = 0.5$). The value of the $\beta$ parameters depends on the position of the gate relative to the DTOF. The variation of $\beta$ along the gates can be explained by the fact that $\beta$ depends on the path length distribution $P(s)$ (T. Bellini M. G., 1991), and applying gates at different positions modifies the path length distribution because we are considering different parts of the DTOF. The high value of $\beta$ is possible thanks to the high coherence of the light source, and because of the use of narrow gates, that weaken the requirement on the source coherence (see Section 1.3).

After having computed the autocorrelation functions, we have fitted them with a single exponential function, to retrieve the value of the decay constant $k_s$ (see Chapter 3). Finally, we have fitted the $k_s$ points with a line, considering only the gates belonging to a 500 ps window that starts from 80% DTOF falling edge. Figure 6.3 shows the retrieved decay rates $k_s$. From Figure 6.3 is it possible to see that the decay rates $k_s$ are well disposed along a line as expected by the theory. In addition to that, the slope of the $k_s$ points increase for increasing reduced scattering coefficient. In fact, the theory predicts that the slope is proportional to $\mu'_s D_B$. Figure 6.3 shows also the light source intensity profile over time. The intensity fluctuations of the source are probably the reason why not all the $g_2$ curves decay exactly to one.
Figure 6.3: Upper plot: narrow gates (100 ps) autocorrelation decay rates $k_s$ (diamonds) and linear fits (continuous lines) for the three phantoms (black, red and blue). The $k_s$ points are well disposed along a line, and their slope increases for increasing $\mu'_s$, as expected by the theory. Lower plots: light source intensity profiles, with a resolution of 1 s, for the three phantom experiments.

Figure 6.4 shows the retrieved value of the rBFI (BFI normalized to its average) for the three phantoms.

Figure 6.4 shows the retrieved value of the rBFI (BFI normalized to its average) for the three phantoms. The rBFI is constant for varying reducing scattering coefficient, except for the first phantom, probably due to the larger laser fluctuations during the measurement (see Figure 6.3).

We can see from Figure 6.4 that the retrieved value of the BFI does not depend strongly on the considered phantom. This is an indication that, at least in a homogeneous medium, we are able to correctly disentangle variations in the optical parameters from variation in the BF.
In Figure 6.5 I show a comparison between narrow gates and ungated acquisition values of the $\beta$ parameter, for the three phantoms considered.

![Figure 6.5: comparison between the value of beta for narrow gates (diamonds) and for ungated acquisition (dashed lines). Black, red and blue colours correspond to $\mu'_{\text{s}} = 5,6,7 \, \text{cm}^{-1}$, respectively. We can see that, for the narrow gates, the value of $\beta$ starts from a value higher than the one for ungated acquisition, but then it decreases gradually below it.](image)

Figure 6.5 shows that for times at which the DTOF is sufficiently high (before it falls below $10^{-1} - 10^{-2}$ its maximum) the value of $\beta$ for narrow gates is bigger than the one for ungated acquisition, for all the phantom considered. This is expected, since in ungated acquisition the span of path lengths is much bigger and thus the coherence requirement is more difficult to fulfil. However, it is important to note that the SNR increases with the beta parameter but also with the count rate, which in ungated acquisition is higher since we are collecting all the photons in the DTOF. Thus, we will see that making larger gates, even if reduces the beta, increases the count rate, and permits to obtain a sufficiently high SNR with the light source at our disposal.

6.2 - Phantom experiment: changing wavelength

The second TD DCS experiment we have carried out was still done on liquid phantoms, but this time changing the wavelength of the light source. We have considered three different wavelengths: $\lambda = 702, 745, 785 \, \text{nm}$. Changing the wavelength of the laser is possible by tuning the filter which is present in the cavity. Figure 6.5 shows the measured DTOF for the three phantoms considered, together with the measured IRF.
Figure 6.6 suggests that the optical properties of the phantom change with the wavelength. Typically, to model the wavelength dependent reduced scattering coefficient $\mu'_s(\lambda)$ one uses the so-called Mie Model:

$$\mu'_s(\lambda) = c\lambda^{-b}$$

(6-1)

Where $c$ and $b$ are constants determined empirically by fitting the $\mu'_s(\lambda)$ for several wavelengths. For our phantoms, the value of those parameters has been determined previously by other colleagues ($\mu'_s = 7 \text{ cm}^{-1}, b = 2.93, \lambda_0 = 785 \text{ nm}$). Figure 6.7 shows the retrieved narrow gates intensity autocorrelation functions for 130 ps gates with 50 % overlap, for the three considered wavelengths.

We see from figure 6.7 that the intensity autocorrelation curves show a sufficient SNR for all the three wavelengths considered. Changing wavelength does not degrade the value of the $\beta$ parameter. This can be an indication that the light source at our disposal could be, in principle, used for TD DCS measurements at different wavelengths. Figure 6.8 shows the retrieved decay rates $k_s$, together with their linear fits, and the light source intensity profile.
Figure 6-8: Decay rates $k_s$ for the liquid phantom and light source intensity profile, for the three wavelengths considered. Note how the linear fit of $k_s$ have all the same $x$-intercept (at $t \approx 1.5 \text{ ns}$).

As it is possible to see from Figure 6.8, the decay rates $k_s$ are well disposed along a line for all the three wavelengths considered, with a slope depending on the wavelength (and thus on the values of the reduced scattering coefficient and of the wave-vector). For very early times we see that, as for the previous experiment, the decay rates $k_s$ deviate from the linear trend. This phenomenon has been studied theoretically in detail in Chapter 5, and can be attributed to non-diffusive and IRF effects on the autocorrelation functions. It is important to notice that the linear fits of the decay rates have the same $x$-intercept (at $t \approx 1.5 \text{ ns}$), a time very close to the peak of the IRF. This confirms that it is possible to use the time at which the IRF has its peak to estimate $t_0$. In Figure 6.8 I show a comparison of the BFI retrieved with this method (narrow gates) and ungated acquisition.
One can see from Figure 6.7 that the value of BFI retrieved with the narrow gates is reasonably similar to the one retrieved with ungated acquisition for all the wavelengths considered. The non-perfect agreement can be because in the measurement the width of the narrow gates is always finite for keeping the count rate sufficiently high (so the narrow gates autocorrelation functions are not single exponentials), to non-diffusive effects (i.e. photons with small number of scattering events) and to the fact that we are not correcting for IRF effects. Figure 6.10 shows a comparison of the value of $\beta$ for narrow gates and for ungated acquisition.

Also in this experiment, we see that the values of $\beta$ are sufficiently high even in the case of ungated acquisition. We will see later in this chapter that this enables us to measure autocorrelation functions with a sufficient SNR even in the case of broader gates, where the coherence requirement is more difficult to fulfil.
This experiment shows that the setup can measure gated autocorrelation functions, with a sufficient SNR, for different wavelengths. This could permit, for instance, to make simultaneous measurements of BF and constituent concentrations (for instance oxy- and deoxy- haemoglobin).

6.3 - Layered phantom experiment

Up to now we have considered media that are dynamically homogeneous. Next, we have carried out an experiment on a system composed of two layers with different dynamical properties (see Figure 6.12). The layer closer to source and detector fibers will be called Superficial layer (S), while the one more distant will be called Deep layer (D). The two layers are separated by a 50 μm thick Mylar sheet.

We have carried out the experiment for three different values of the thickness δ of the superficial layer, δ = 5, 10, 15 mm. The thickness of the deeper layer (around 10 cm) was high enough to mimic a semi-infinite slab. The SD separation was set to ρ = 15 mm for all the experiments. First, we prepared a liquid phantom made of lipofundin and water. Both the tanks were filled with this first phantom. After, we substituted the phantom of the superficial layer with a second phantom with 30 % glycerol in weight, keeping in the deeper layer the first phantom. The addition of glycerol increases the viscosity of the liquid and thus decreases the Brownian diffusion coefficient $D_B$. In this experiment we want to understand whether the TD DCS setup can resolve depth-localized changes in the mobility of the scatters (and thus in the BF).

First, we have verified that the receipt used for the two mixtures does not change the reduced scattering coefficient. This was done placing the two phantoms in another tank and doing a TD NIRS measurement, as illustrated in Figure 6.12.
The retrieved values of $\mu'_s$ for the homogenous mixtures without and with glycerol was 10.5 and 10.6 $cm^{-1}$. The absorption coefficient is slightly changing between the two mixtures, but the decay rate $k_s$ is insensitive to absorption and thus we can neglect this fact. After that, we proceeded with the actual layered phantom experiments, in which we considered the three different thicknesses and the two different systems: dynamically homogenous (0 % glycerol in deeper and superficial layer) and dynamically heterogeneous (deeper layer 0 % glycerol and superficial layer 30 % glycerol).

**Narrow gates**

We computed the autocorrelation functions considering narrow gates of two different widths, 100 $ps$ and 160 $ps$, also to check if the retrieved value of the BFI depends on the choice of the width of the gates (in any case remaining in the narrow gate limit). Then we have calculated the decay rates $k_s$ for each gate and computed the BFI. The BFI was computed considering in the linear fit of the $k_s$ points belonging to two different regions:

- Early photons: $k_s$ between 80 % DTOF rising edge and 80 % DTOF falling edge
- Late photons: $k_s$ between 50 % DTOF falling edge and 10 % DTOF falling edge

Note that we have left a temporal window between the end of the early and the start of the late regions, to reject the transition between the two. The following Figures (6.12-6.13) show the measured decay rates $k_s$ in the case of 100 $ps$ and 160 $ps$ gates. The dynamically homogenous system was measured only for $\delta = 5,10 $ mm.
Figure 6-13: Computed decay rates $k_s$ for the bilayer system experiment, 100 ps gates. The open diamonds and filled circles refer to the dynamically homogeneous and dynamically heterogeneous (00 % and 30 % glycerol phantom in the superficial layer, respectively). The deeper layer was always filled with the 00% glycerol phantom. Blue, red and black correspond to thickness of the superficial layer $\delta = 5, 10, 15$ mm. The dashed vertical lines enclose the $k_s$ points that have been considered in the linear fit for computing the early and late BFI.

Figure 6-14: Computed decay rates $k_s$ for the bilayer system experiment, 160 ps gates. Note how the $k_s$ points are less noisy compared to the 100 ps gates case.

We can see from the previous Figures that the computed decay rates $k_s$ does not depend on the gate width, as expected in the narrow gates limit, even if with a smaller gate width the decay rates $k_s$ are noisier due to the lower count rate. Consequently, using a bigger gate width (for now remaining in the narrow gate limit) can give us a more reliable estimation of the BFI. Table 6.1 shows the retrieved values of the BFI for the early and late regions, in the cases of 100 and 160 ps gates.
We can see from the previous tables that the ratio between early and late BFI decreases when we pass from the dynamically homogeneous to the dynamically heterogeneous systems. This is expected, because the early photons probe a more superficial region compared to late photons, and thus they are more sensible to the superficial flow. We see that also the late photons BFI decreases, but by a smaller amount, when we add the glycerol. This is because, even if late photons probe deeper regions compared to early photons, they still need to pass through the superficial layer, and thus they are also slightly affected by the superficial flow. We must note that a complete disentanglement between superficial and deeper regions is difficult to obtain also in TD NIRS. In addition to that, we see that, even in the dynamically homogenous case, the late photons BFI is larger than the early photons BFI. As already discussed before, we believe that this is mainly because we are not compensating for the effect of the IRF on the autocorrelation functions (see Chapter 3.3 and 5).

Thus, we can see that we are able to detect depth localized changes in the particles’ motion by analysing the gated autocorrelations’ decay rates at different temporal regions. In the next section I will show that this method is difficult to apply in in-vivo measurements, due to its limited SNR, and I will show that broader gates (see Section 3.2) give better autocorrelation functions and BF changes estimates.
6.4 - Head changing applied pressure experiment

After the phantom studies, we have passed to *in-vivo* studies to see if TD DCS has a potential practical application to human studies. To this extent, we have done TD-DCS measurements on the head of an adult subject. We have selected one healthy subject, and we have placed our probe in the frontal part of the head. We have asked to our subject to lie comfortably on a bed. The protocol selected consisted of two parts: 3 minutes of baseline and 3 minutes of manual pressure on the head of the subject. The pressure on the head has been applied to study whether the BF was sensible to external changes. We expect the superficial blood flow (extra-cerebral BF) to be more sensible than deep blood flow (CBF) to this kind of non-invasive manipulation.

Narrow gates

As for the layered phantom experiment, we have started our analysis using narrow gates. In this experiment we have considered gates with temporal duration of 100 ps and 130 ps. The light source was attenuated to meet the Maximum Permissible Exposure limit for skin that, according to our calculations based on the ANSI standards, was 30 mW. After having computed the narrow gates autocorrelations for the two cases of no pressure and pressure, we have fitted the decay rates $k_s$ considering three regions:

- *Early* photons region: considering $k_s$ between 80 % DTOF rising edge and 90 % DTOF falling edge
- *Late* photons region: considering $k_s$ between 80 % DTOF falling edge and 50 % DTOF falling edge
- *Single* region: considering $k_s$ between 80 % DTOF rising edge and 50 % DTOF falling edge

The end of the late region was chosen based on the SNR of the autocorrelation functions (and correspondingly of the decay rate). In Figure 6.15-16 I show the measured DTOF and computed decay rates $k_s$ (for gates with 100 ps and 130 ps width), for the two cases of no pressure and pressure applied.
Figure 6-15: head experiment, no pressure. Upper plot: measured DTOF, together with the limits of the early and late regions (single fit was carried out from the start of the early region to the end of the late region). Lower plot: computed decay rates $k_s$, for 100 ps and 130 ps gates (blue diamonds and red circles, respectively).

Figure 6-16: head experiment, with pressure applied.
The resulting early, late and single fit BFI are resumed in Table 6.3, for the two measurements and in the case of 100 ps gates. We assumed a reduced scattering coefficient of $\mu'_s = 10 \text{ cm}^{-1}$ for all the cases. Also, the values of the rBFI for the early and late fits (normalized to the single fit, no pressure condition) are shown.

Table 6-3: Table of the computed values of $D_B$ using 100 ps gates, for the three cases of early fit, single fit and late fit. Also, rBFI for the early and late regions (normalized to the single fit, no pressure condition) are shown.

<table>
<thead>
<tr>
<th>$D_B$ (10^{-9} \text{cm}^2/\text{s})$</th>
<th>$rBFI$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_B$ Early fit</td>
<td>$D_B$ Single fit</td>
</tr>
<tr>
<td>subject 02 no pressure</td>
<td>3.68</td>
</tr>
<tr>
<td>subject 02 with pressure</td>
<td>0.556</td>
</tr>
</tbody>
</table>

We can see from Table 6.3 that, when pressure is applied, the early fit $D_B$ decreases significantly. On the other hand, also single fit and late fit $D_B$ decrease. The reduction in the early $D_B$ is expected because the applied pressure affects mainly the superficial regions like scalp and skull. The reduction in late $D_B$ can be explained by the fact that, even if for longer path lengths the photons are reaching deeper regions, the photons still need to pass through the more superficial layers. Thus, variations in the superficial flow will always give rise to variations also in the late $D_B$. On the other hand, we note that the factor of decrease of the early BFI (6.5) is bigger than the factor of decrease of the late BFI (4.8), as expected.

We must point out that the resulting decay rates $k_s$ are quite noisy, thus the retrieved BFI are affected by some uncertainty due to the low SNR. For this reason, we have passed to the use of broader gates for increasing the SNR of the autocorrelations and, correspondingly, the reliability of the BFI estimates. In the rest of the Chapter, I will show that broad gates are a more suitable method for estimation of the BFI in-vivo measurements.

**Broad gates**

In order to increase the SNR, we passed to a broad gates analysis. We have considered two broad gates, early and late, in order to see wether we can differentiate superficial and deeper BFI changes. In addition to that, we have also considered ungated acquisition. The limit of the gates was chosen as follows:

- **Early gate**: from the IRF peak to 90 % DTOF falling edge,
- **Late gate**: from 90 % DTOF falling edge to the DTOF noise floor,
- **Ungated**: from the IRF peak to the DTOF noise floor.

Figure 6.17 shows the three measured autocorrelation functions for the case of no pressure applied.
Figure 6-17: Head experiment, broad gates autocorrelation functions for the no pressure measurement. Note how the $\beta$ parameter depends on the considered autocorrelation. For all the three cases, the $\beta$ parameter is high, almost comparable to the “standard” DCS value of 0.5. Note the different decay rates of the three autocorrelations.

We can see that the computed autocorrelation functions show a high value of the $\beta$ parameter ($\beta = 0.41, 0.29, 0.25$ for early, ungated and late autocorrelations respectively). This is an indication that the use of a very coherent pulsed light source enables a sufficient value of $\beta$ even for the case of broad gates and ungated acquisition). This enables higher count rates within the gate and thus bigger SNR. In addition to that, as expected (see Chapter 3 and 5), the autocorrelations show different decay rates. This is a mixed contribution of different blood flows $D_B$ in the various head’s layers, but also different path lengths $s$, in fact the decay rate of the single path autocorrelation function $g_{1,single}(\tau)$ is proportional to the product $D_B s$.

Figure 6.18 shows a comparison between the no pressure and pressure measurements, in the three cases of early gate, ungated and late gate. The fits have been carried out using the integral expression of the $g_1$ (eq. 3.26), considering only points from $\tau = 10^{-5}$s (to reject after pulsing) to the lag time $\tau$ at which $g_1 = 0.5$ (to reject the noise and normalization artefacts at high correlation times). Note the different decay rates of the autocorrelation functions passing from no pressure to pressure condition (for a fixed gate), indicating a different BF, and the rather good fit quality for the points considered in the fit (i.e. $g_1 \geq 0.5$).
Using the $D_B$ retrieved from the autocorrelations fit, we have computed an $rBFI$ index for early and late gates by normalizing the $D_B$ of each gate (early and late) to the $D_B$ retrieved with ungated acquisition, in the no pressure condition. Figure 6.19 shows the resulting rBFI for the two conditions of no pressure and pressure applied.

We can see from Figure 6.19 that both the early and late rBFI are decreasing when pressure is applied. Furthermore, the difference between early and late rBFI increase when applying pressure, as expected since early photons are more sensible to the decrease of superficial blood flow induced by the pressure. The decrease in late rBFI is because, even if the late photons
reach deeper regions, they need in any case to pass through the upper layer. Thus, a complete
disentanglement of superficial and deeper flow is difficult to obtain. In any case, the relative
decrease in the late rBFI is much smaller than the relative decrease in the early rBFI.

This result show that the use of broad gates gives more consistent estimations of variations in
the BFI. This method is applicable only with the use of highly coherent pulsed light sources.

6.5 - Cuff occlusion experiment

For testing the ability of TD DCS to recover dynamic changes in the Blood Flow, we have done
a cuff occlusion experiment, on the forearm of a healthy subject. The protocol was 4 minutes
of rest, 3 minutes of cuff occlusion and 3 minutes of recover. We placed our probe in contact
with the tissue and selected a SD separation $\rho = 1.55 \text{ cm}$.

To compute the BFI at different times during the experiment, we have divided our photon
stream in windows of 10 s and computed the gated autocorrelation functions for each of those
windows. This functionality can be called “macro-time” gating. Basically, the difference
between path length gating and macro-time gating is this one: the path length selects the
spatial regions of the tissue that we want to investigate, the macro-time select the temporal
window that we want to consider. For instance, we can discriminate between superficial/deep
tissue with path length gating, and between occlusion/recovery with the macro-time. The
following Figure shows the DTOF, acquired cumulating all the photons detected in the
experiment, and the IRF.

![Figure 6-20: TD NIRS fit for the cuff occlusion experiment. For simplicity, we have computed the DTOF considering the whole time window of the experiment and then fitted it with a semi-infinite homogenous model. The fit quality at the tail is not high, probably due to changes in the absorption coefficient during the experiment.](image)

We can see from the previous Figure that the TD NIRS fit obtained using a semi-infinite
homogeneous model does not fit perfectly the DTOF at its tail. This can be due either to
spatial inhomogeneity of the probed tissue (skin, fat and muscle layers) or to temporal variations in the optical properties of the tissue (due to the cuff occlusion). A more accurate approach should be to consider a multi-layer model to fit the data, and to compute the value of the optical parameters as a function of time.

**Broad Gates**

To keep the SNR sufficiently high, we have resorted to a broad gates analysis of our data. To do so, we have computed the early gate, ungated and late gate autocorrelation function for each of the 10 s windows in which we divided the experiment. This method permits both to have spatial (i.e. depth) resolution and temporal resolution, as already pointed about before. Each of the computed autocorrelation curves was then fitted with the integral model. To compute \( P(s) \), we have used a semi-infinite homogenous model and substituted the values of the optical parameters obtained with the TD NIRS fit. Only points with correlation time > 1E-5 s and \( g_1 > 0.5 \) have been considered in the fit, to reject after-pulsing (at low lag times) and noise/normalization artefacts (at high lag times). In the evaluation of the integral, the extremes of integration have been rescaled by \( t_0 \) (the peak of the IRF) to account for the fact that the \( P(s) \) is always centred in the origin (being a path length distribution). The following Figure shows the computed autocorrelation curves and the corresponding fits for three moments of the measurement: before the occlusion, during the occlusion and at the hyperaemic peak (determined considering the time at which the rBFI reached its maximum).

![Figure 6-21: broad gates autocorrelation curves before, during and after (at the hyperaemic peak) the cuff occlusion. The open circles and the continuous lines are the measured intensity autocorrelations and the fits. The](image-url)
three plots correspond to early gate, ungated acquisition and late gate. Only points with correlation time > 1E-5 s and $g_1 > 0.5$ have been considered in the fit to reject after-pulsing (at low lag times) and noise/normalization artefacts (at high lag times).

We can see from the previous figure that, in all the three gates considered, the autocorrelation curve shows different time decay according to the physiological condition. During the occlusion the autocorrelation decays much slower (suggesting a smaller flow), and after the occlusion it decays faster with respect to the original (i.e. baseline) decay rate. We can see that the use of the integral model for the fit returns quite good fit quality for all the gates, and all the physiological conditions. We note that the value of $\beta$ is very high (~0.4), almost equal to the value expected with a steady-state source (0.5), even if we are using broad gates. This is possible because we are using a very coherent laser as light source. The following figure shows the retrieved rBFI, normalized to the first 200 s of measurement, for early, late and ungated acquisition.

![Graph](image)

*Figure 6-22: computed rBFI for the cuff occlusion experiment, normalized to the first 200 s of measurement. The vertical dashed lines correspond to the starting and releasing time of the cuff occlusion. The horizontal dotted line corresponds to the baseline (i.e. rBFI = 100%). As expected, the BFI decreases immediately after the occlusion and have a strong overshoot (hyperaemia) after the release. The three rBFI show a similar temporal behaviour. The hyperaemic peak is slightly higher in the late gate.*

We can see from the previous Figure that in the early, ungated and late cases, the rBFI shows a similar temporal behaviour. Before the occlusion, the rBFI remains constant, and then it drops when we start the occlusion. After roughly 50 s from the release, the rBFI shows a strong peak, the so called hyperaemic response. During the baseline and the cuff occlusion the three rBFI show a similar behaviour, while the late gate shows a slightly larger rBFI with respect to the other two cases. This could be an indication that we are able to see that the deeper layer (muscle) has a stronger auto-regulatory response with respect to the superficial layer (skin and fat), but more subjects need to be analysed to confirm that.
6.7 – Conclusions

In this Thesis work I have focused on a very new technique of Diffuse Optics: Time Domain Diffuse Correlation Spectroscopy (TD DCS). First, I have shown how it is possible to build a TD DCS setup, with the use of a pulsed light source with very high coherence and a TCSPC light detection system coupled with a Software Correlator. The key aspect of TD DCS is that, since it uses a pulsed light source instead of a steady-state one, is possible to measure a depth-resolved Blood Flow. The source that we used permitted us to exploit the technique for in-vivo human studies, for instance for the study of the human brain under different physiological conditions. Additionally, the temporal resolution of our measurements was approximately 1 s. This fast temporal resolution permitted us to apply the technique to dynamically-changing measurements such as a cuff occlusion on the arm of a human subject. Since TD DCS is a particularly new technique, various “non-ideal” effects such as low number of scattering events and finite source-detector temporal resolution needed to be studied in detail. For this reason, during my work I reviewed the existing theory for TD DCS and tried to extend it for considering these effects. The use of Monte Carlo simulations helped me to quantify these effects and to study the accuracy of the proposed models. In conclusion, in this thesis I have shown both experimental and theoretical methods that can be used in TD DCS, and that can make this technique effective for practical use.
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