**Master in Photonics**

**MASTER THESIS WORK**

**DESIGN AND CONSTRUCTION OF A PHOTOACOUSTIC IMAGING SETUP**

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Design and construction of a photoacoustic imaging setup

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Abstract. Photoacoustic Microscopy (PAM) is a novel imaging technique that is commonly used with samples composed by soft tissue and that can provide penetration beyond the optical diffusion limit while maintaining high resolution. By using a short-pulsed laser to irradiate the tissue, part of the light energy is converted into heat, which induces the formation of acoustic waves that propagate inside the tissue and can be detected through ultrasound transducers. In this way, the absorption coefficient can be used as a contrast property of the matter, allowing the mapping of biological and chemical information. The essence of this master thesis is the design and start up of a PAM setup. This report contains the explanation of the different steps performed before reaching this goal, together with a justification of the different decisions that were taken along its construction. Finally, the resulting device and its main features are shown and discussed.

Keywords: photoacoustic microscopy, ultrasound, acoustics, imaging, instrumentation

1. Introduction

Photoacoustics is an emergent field that started being used during the last decade[1] in various research disciplines, including chemistry[2], material science[3] and biomedicine[4]. The operational steps of photoacoustic imaging are simple: when intense pulsed light, commonly coming from a laser, impacts on the target, a fraction of the light is absorbed, resulting in the transformation of part of the energy into heat. The latter generates a peak of pressure that propagates as an ultrasonic acoustic wave, and thus this acoustic pulse can be detected by means of ultrasound transducers. Moreover, a 3D image can be constructed by using scanning techniques, reconstruction algorithms[5] and the time-of-flight principle[6]. This simple method opens a broad spectrum of potential setups that can be applied to a large variety of applications for detection purposes. Tuning the number and type of transducers, the wavelength of the incident light and the spot size of the laser beam, among others, might result in different scanning features. One of these modalities is photoacoustic microscopy (PAM)[7, 8], which is characterized by a very good resolution at the price of a relatively poor penetration and a slow scanning time[9]. Structures with high optical absorption, such as blood vessels[10] or regions with a high concentration of melanin[11], can be imaged with high ultrasonic resolution and without the strong light scattering limitation inside the medium, which is the main cause for low penetration depth in traditional optical techniques.

This Master Thesis is focused in the design, construction and set up of a functional and compact PAM setup in the Center for Sensors, Instruments and Systems Development (CD6). In order to offer a comfortable use of the PAM setup in the future, a simple user guide and an intuitive control software is also developed, together with
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some improvements with the intention of ease the adaptation of the PAM in other more specific or demanding structures. The development of the PAM setup will allow this research center to get started into the growing field of photoacoustic imaging just at the moment when this technology starts being implemented in commercial devices. One of the motivations to develop this Master Thesis has been to focus not only in the research work but also in the initiative, technical work and creativity that implies the fact of starting a new research line. Either to advance in the implementation and improvement of photoacoustic technology or to combine PAM with other imagine techniques, this Master Thesis intends to be a starting point and an important tool in the difficult way of transforming scientific knowledge into helpful technology.

2. Fundamentals of photoacoustic microscopy

2.1. Photoacoustic signal generation

In order to obtain a relation between the absorbed energy of the light and the generated pressure wave, a model from a thermodynamic point of view is invoked[12]. In the ANNEX, the detailed mathematical and physic model about the transformation from light pulse to ultrasound waves is developed. It results in the next reduces wave equation

\[
\left( \frac{\partial^2}{\partial t^2} - v_s^2 \nabla^2 \right) \delta P = \Gamma \frac{\partial Q}{\partial t}
\]  

(1)

where \( \delta P \) refers to the pressure perturbation in tissue, \( Q \) is the heat produced by the pulse laser, \( v_s \) is the speed of propagation of the resulting wave and \( \Gamma \) is a dimensionless constant called the Grüneisen parameter, which indicates the efficiency of conversion of absorbed energy, i.e. heat, into pressure. Now we can assume that the temporal dependence of \( Q \) can be approximated by a delta function since the heating pulse occurs on a timescale much shorter than the characteristic acoustic travel time. In this way, the resulting excited wave equation (Eq. 1) becomes an initial value problem:

\[
\delta P_0(r) = \Gamma Q(r)
\]  

(2)

Therefore, the resulting dynamics is a propagation and dissipation of these initial values. The maximum value of pressure perturbation will be \( \delta P_0 \), hence proportional to the spatial distribution of heat generation. This allocation of heat depends on the light intensity \( \Phi(r) \) by

\[
Q(r) = \mu_a(r) \Phi(r)
\]  

(3)

where \( \mu_a \) accounts for the optical absorption coefficient, which can be briefly defined as the capability of the tissue to absorb photons and generate heat. Hence \( \mu_a \) is basically the contrast property of the sample and the intensity of the ultrasonic wave will be proportional to photon flux. The absorption coefficient is dramatically dependent on the wavelength used in the laser beam, thus photoacoustic imaging can be implemented with a tunable laser in order to offer spectroscopic information[13].

2.2. Immersion focused ultrasound transducers

The study of the propagation of the acoustic waves from the mentioned initial pressure distribution is an important step because the ultrasonic transducers are typically away from these sources. Complex algorithms can be necessary to recover the information in order to image \( \mu_a(r) \), but it is not the case of PAM because it uses a specific type of transducer.
Figure 1. The focused ultrasound transducer basically detects signals only from the focal zone ($F_z$), which has a cylindrical shape and is centered in the so-called focal point. $Z_B$ and $Z_E$ refer to the beginning and end of the focal zone respectively and $d$ accounts for the diameter of the focal zone.

A transducer is any device that converts one form of energy into another. Hence, an ultrasonic transducer converts electrical energy into acoustic energy and vice versa[14]. Each ultrasonic transducer works in a specific region of the spectrum, meaning that the central frequency ($f$) of this region is an important feature of the device. Each transducer has a certain active area which is defined as the surface that emits or receives the acoustic wave. It is usually circular, consequently a cylindrical geometry is commonly considered. Although we can find a great variety of possible transducers, only one type is optimized for samples composed by soft tissue: immersion transducers. These kind of transducers do not require direct contact and are more sensitive to low signals. Besides this, in the case of PAM we need to select an additional feature called focusing.

In Figure 1 the concept of focused transducer is sketched. The electrical signal produced by the ultrasound wave from a certain point (from now named focal point) is amplified by using acoustic lenses. This focal point is in the so-called maximum intensity line which is defined in the axial direction from the center of the active area. Following this line and near the focal point we can define the focal zone ($F_z$): the cylindrical region where an acoustic wave source can be detected by the transducer. Therefore some important parameters are defined in the focused transducer: the diameter of the active area ($D$), the beginning ($Z_B$) and end ($Z_E$) of the focal zone, and its focal length ($F$). These parameters define the focal zone diameter ($d$) using[14]:

$$d = \frac{Fv_s}{fD}$$  \hspace{1cm} (4)

where $v_s$ is the speed of sound. Non-focused transducers cannot differentiate waves from different point sources that are at the same distance (but come from different directions), hence a large number of transducers and complex algorithms are necessary to create an image. In contrast, because PAM relies on focused transducers, scanning the sample and applying the ‘time-of-flight’ principle is enough to properly map the sample.

2.3. The 'time-of-flight' principle

When the laser produces the acoustic wave in tissue, it starts to travel and ends up reaching the active element of the transducer. Therefore, we can determine the distance ($L$) between the transducer and the acoustic source by measuring the time delay ($\tau$) between the moment of the excitation by the laser and the moment when the transducer detects the signal:

$$L = v_s \tau$$  \hspace{1cm} (5)

This technique is called ‘time-of-flight’ and is commonly used in a large variety of range detection devices[6]. In the case of PAM, it allows to generate 3D-images of the sample.
2.4. Generic structure and instrumentation of the PAM setup

The main devices in a PAM setup are the laser and the transducer, but additional instrumentation is also required in order to coordinate the use of both. An oscilloscope is required to read the electronic signal generated by the transducer. This oscilloscope needs to start reading the signal when the laser emits the pulse, hence a trigger must be implemented. A photodiode with a good time response is the more common and suitable way to determine this moment.

The signal generated by the transducer is usually too low, thus a slow noise amplifier is also required. In order to scan the sample, a motion controller is also necessary. Finally a computer and a specific software should control all the devices in a coordinate way and process the data from the oscilloscope to generate the images. This scheme is showed in Figure 2.

2.5. PAM features: resolution and penetration depth

PAM can be implemented in two different modes of operation[9] that define its lateral resolution (resolution in the direction perpendicular to the axis): Optical Resolution Photoacoustic Microscopy (OR-PAM)[15] and Acoustic Resolution Photoacoustic Microscopy (AR-PAM). In the OR-PAM the laser beam is focused at the focal point of the transducer with a spot diameter lower than the diameter of the focal zone. This kind of PAM has a very poor penetration depth due to the large scattering of light in soft tissues, which prevents the focusing goal. In contrast, in AR-PAM, the lateral resolution is fixed by the diameter of the focal zone of the transducer, thereby the laser beam does not need to be focused which benefits penetration depth.

The axial resolution ($R_{axial}$) of any PAM configuration is, on the other hand, basically determined by the capability to differentiate two pulses in the focal zone of the transducer. This depends on the bandwidth of the transducer ($\Delta f$) as follows[16]:

$$R_{axial} = \frac{0.88v_s}{\Delta f}$$  \hspace{1cm} (6)

Regarding penetration depth, even though the scattering of the incident light is the main limitation it is not the only one. When working with high ultrasound frequencies like the ones commonly used in PAM the acoustic absorption of the medium cannot be neglected (as was intrinsically assumed when we modeled the soft tissue as an inviscid
fluid in the annex). In fact, the acoustic wave vanishes in a few millimeters[9]. This absorption phenomenon mainly depends on the square of the frequency, meaning that in PAM there will always exist a trade-off between the lateral resolution, which depends on the frequency as shown in Eq. 4, and the penetration depth.

3. CD6-PAM setup

3.1. Determination of the typology

The original goal of this thesis was based in the design and construction of a generic photoacoustic setup for CD6 (CD6-PAM). Therefore the first task was to specify the kind of setup to build. In order to know the options and their particular features, some research on the state of the art in photoacoustics was developed. From this, PAM, and in particular AR-PAM, emerged as the best option taking into account the different research lines of CD6. This technique has a perfect adaptation in the melanoma characterization devices and potential to be implemented together with other techniques such as confocal microscopy[17] and optical coherence tomography[18], which are part of the know-how of CD6. Another important reason to select PAM is its reduced cost. Alternatives to PAM require more than one transducer, being this the main part of the cost, except for the pulsed laser. Moreover, in these kind of setups the relative position between the transducers has to be fixed, hence the shape of the sample is also limited.

The arrangement of the AR-PAM is another relevant decision because it can be implemented in the so-called reflection, transversal or transmission mode[19, 20], as schematically sketched in Figure 3. Although reflection mode is the optimal choice[9], the use of an axicon hindered too much the design of the first prototype and consequently this mode was discarded. Similarly, transmission mode was preferred instead of transversal mode because it allows to keep the same axial direction for both the transducer and laser beam, which simplifies the alignment procedure.

3.2. Looking for components

The optimization in the investment was an important motivation in the search of components. It was not necessary to buy a laser because CD6 had already available a diode pumped Q-switching solid-state laser (MQO ARTIC Photonics) working at 1064 nm
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with a pulse duration of 1.5 ns and 20 µJ of energy pulse at maximum repetition rate of 10 kHz. Using a KDP crystal, the pulse is transformed to 532 nm, which is the wavelength at which hemoglobin becomes the contrast agent. The parameters of this laser are close to the common parameters used in published PAM setups[7, 8, 10, 11, 20].

The selection of the focused immersion transducer was based on its central frequency, which determines both the lateral resolution of AR-PAM and the penetration depth. Taking into account that the future use of this setup at CD6 will be its implementation with optical techniques or an improvement in skin inspection, the central frequency must be maximized. This criterion gives CD6-PAM a better penetration depth compared to completely optical techniques and a similar lateral resolution. Lower frequencies offer unnecessarily high penetration depths and poor resolution that generates an image much worse than the ones generated by optical methods. Therefore, 50 MHz was the final choice, since higher frequencies were unaccessible due to the elevated cost of the device. Considering these specifications the Panametrics-V390 was finally acquired. This focused immersion transducer has a central frequency of 44.52 MHz, Δf of 37 MHz, F of 12.7 mm and D of 6.3 mm.

Taking into account the frequencies reached by the electronic signal generated by the transducer, a correct amplifier for radio frequencies was bought (Mini-Circuits ZFL-500LN). In addition to this, the motorized motion controller to scan the sample is a XYZ-assembly of three linear actuators (Zaber X-LSM050A) with 47 nm of resolution and a travel range of 500.8 mm. The oscilloscope (Tektronix DPO 2024), the function generator (Hameg HM-8150) that control the laser and the voltage supply were already available at CD6.

3.3. Mechanical design and setting-up

The mounting structure was built using only Thorlabs standard pieces. In order to justify the Thorlabs purchase, a mechanical design was developed using the commercial software Geomagic Design (old Alibre) and the STEP files given by the supplier. Figure 4 compares this 3D design to a picture of the final CD6-PAM. The support of the laser, sample holder and tank of water were designed using the same software and built at the mechanical workshop of CD6. A focusing lens is placed in front of the laser to focus the beam in the focal point of the transducer.

The complete setup is mounted on an aluminum breadboard of 45x45 cm² and the transducer is hold up by a XYZ translation stage (Thorlabs 150-811ST) in order to do the micrometric alignment between the optical and acoustic axis. The photodiode that sends the trigger signal to the oscilloscope is placed on the laser support.

3.4. Full-Labview control and visualization software

With the purpose of allowing an easy use of the CD6-PAM, the control of all the instrumentation and processing data is unified in a single program in a LabView environment. Figure 5 shows the control interface, where letter labels are used in order to help the explanation in this report. The first step when using CD6-PAM is to calibrate the oscilloscope with the signal generated by the transducer and to align the transducer with the laser beam. An auto-calibration process of the oscilloscope is also implemented in the software of CD6-PAM. Every time that the button in A is clicked, the oscilloscope is adapted to the received signals (from photodiode and transducer) and the trigger signal is shown in the graph. Using the button in B, the peak of the photoacoustic signal and the distance to the source are displayed. This is useful when manual alignment of the transducer is needed.
Only when the calibration is done, the motion controller of the sample and the scanning are available. Sections C are devoted to the movement of the sample and the determination of the speed of the displacement. Finally, the scanning part D uses the input data 'Threshold' to determine when a detected peak is a photoacoustic signal. Here the program offers two ways to scan the sample. The first one does discrete steps in the X and Y directions and in each step a transducer signal is read and processed. The processing is based on detecting the peaks of the signal, determining whether it is above the threshold or not, reading the time delay with respect to the trigger and applying Eq. 5 in order to obtain the relative distance between the acoustic source and the transducer. In contrast the second option to scan the sample, the motion controller carries out a continuous passage in the X direction but discrete steps in the Y direction. Data is gathered during the X scans and the number of points of the image is determined by the speed of displacement of the sample and the time spent by the computer and the oscilloscope to read and process a signal. Although the second type of scan should offer a lower scanning time, the mentioned time spent by the system to get and process a signal is greater than the time spent by the motion controller in making one step. Accordingly, the first type of scan is in fact the right choice because it is more robust.

3.5. Images acquired

Below the control interface, three different kind of images are given as the output of the scan: surface profile, absorption map and point cloud. In Figure 6 the three kind of images coming from the same sample, a human brown hair, are shown. In the surface profile, the first photoacoustic peak received in a given transducer signal is read. This information offers a profile of the closest point, i.e., the surface of the sample. The second kind of image, the absorption map, also gets information coming only from the surface but in this case the value plotted is the amplitude of the photoacoustic signals. This can be useful to map different concentrations of contrast agents. Finally, the point cloud is a 3D image of the sample, where each point represents an acoustic source.

4. Characterization of the CD6-PAM setup

The lateral resolution of the CD6-PAM setup is determined using a USAF 1951 resolution negative test target, which is placed at the position of the sample. This negative target is composed by a glass substrate with some areas covered by a chrome coating forming a pattern. Although chrome coating is far from being soft tissue it behaves as a good photoacoustic source[21]. Different patterns, corresponding to lines with different spacing and thickness, were scanned until the smallest detectable feature was found.
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Figure 5. The control interface is composed by well defined sections. A and B are dedicated to auto-calibration. C sections refer to sample motion: C1 moves the sample to a particular position, C2 determines the speed of displacement, C3 sends the sample to the zero position, C4 informs about the current exact position of the sample and C5 performs a particular displacement. D controls the two types of scans.

Figure 6. Respective a) surface profile, b) absorption map and c) point cloud images of a particular brown hair with diameter of 150 µm.

Figure 7 shows the target together with a surface profile image in the minimum resolution zone that the CD6-PAM can detect. It corresponds to an area of the target with 12.7 line pairs per millimeter. The length of these lines is 39.4 µm, a value close to the 67 µm of the diameter of the focal zone of the transducer derived by Eq. 4. Both surface profile or absorption map plots show the same lateral resolution.

The determination of the axial resolution was done by considering the standard deviation of the distance from a single photoacoustic source. Using the same resolution target (in a chrome zone), some measurements were performed using different laser intensities and no variation was found in the shape of the acoustic signal read by the transducer. Taking this into account, a total of 100 measurements with a given laser intensity were done using a new Labview program in order to get the corresponding standard deviation. This resulted in 42 µm, a value close to the 35.2 µm value derived from Eq.6.
Another important feature is scanning time. An additional Labview routine was developed in order to perform an average of the time spent by the CD6-PAM setup to record an image point (including the time of sample displacement). It resulted in a scanning speed of 2.1 seconds per point.

5. Future work

This thesis corresponds to a first prototype of a CD6-PAM, thus there is still plenty of room for improvement. The first crucial step is transforming the arrangement into a reflection mode setup (Figure 3), instead of a transmission one. It will allow CD6-PAM to image thick samples and to operate as a confocal microscope. Along with this change, the oscilloscope must be substituted by a fast digitizer in order to reach an acceptable scanning speed.

6. Conclusions

A functional AR-PAM setup with competitive lateral and axial resolution has been successfully implemented from scratch, including a complete analysis of the theoretical and experimental aspects involved, the selection of components and the characterization of its current performance. Given that it is a first prototype, some limitations such as the elevated scanning time must be solved but concrete solutions have already been proposed. The unified software and automatized control of the instrumentation enables its use to scientists with any kind of background and paves the way for future developments. Moreover, the images containing information such as the acoustic wave amplitude open new doors in biomedical characterization. Taking all of this into account, the initiative, research work and engineering labour of this Master Thesis has resulted in a powerful tool in order to start in the transversal research field named photoacoustics, which promises to be a main topic in the biomedical photonics sector during the next decade.

ANNEX: Photoacoustic signal generation model

Commonly the samples used in this PAM setup are soft tissue, thus the target will be approximated as an inviscid fluid. In addition to this, it is also assumed that the acoustic variations in pressure and density are small compared to the equilibrium values. Taking this into account, we can start the model by considering the linearized conservation
equations of mass, momentum and energy

\[
\frac{\partial (\delta \rho)}{\partial t} = -\rho_0 \nabla \cdot (\delta \mathbf{v})
\]

\[
\rho_0 \left( \frac{\partial (\delta \mathbf{v})}{\partial t} \right) = -\nabla (\delta P)
\]

\[
\rho_0 T_0 \left( \frac{\partial (\delta s)}{\partial t} \right) = \nabla \cdot [\kappa \nabla (\delta T)] + Q
\]  

(7)

where \( \kappa \) refers to the thermal conductivity, \( \rho \) is the density, \( T \) accounts for the temperature and \( Q \) is the heat. The explicit dependence on position and time has been neglected in the notation for simplicity. The index 0 indicates the parameter in the equilibrium state and the \( \delta \) symbol refers to the particular variations generated by the heat excitation. The two first equations can be put together by removing \( \mathbf{v} \), which yields the following relation

\[
\frac{\partial^2 (\delta \rho)}{\partial t^2} = \nabla \cdot (\nabla (\delta P))
\]  

(8)

Now is the moment to write entropy and density fluctuations as function of temperature and pressure fluctuations. These four quantities are connected through an equation of state, which can be given either as an explicit equation, relating \( \rho, P, T \) and \( s \), or in terms of the partial derivatives, assuming entropy and density as a function of \( T \) and \( P \):

\[
\delta s = \left( \frac{\partial s}{\partial P} \right)_T \delta P + \left( \frac{\partial s}{\partial T} \right)_P \delta T
\]

\[
\delta \rho = \left( \frac{\partial \rho}{\partial P} \right)_T \delta P + \left( \frac{\partial \rho}{\partial T} \right)_P \delta T
\]  

(9)

Applying the Maxwell relation \( \partial s/\partial P = \left( m_0/\rho_0^2 \right) \partial \rho/\partial T \), where \( m_0 \) is the mass in the infinitesimal volume in equilibrium, and using the following constants

\[
K_T = \frac{1}{\rho_0} \left( \frac{\partial \rho}{\partial P} \right)_T
\]

\[
C_p = T_0 \left( \frac{\partial s}{\partial P} \right)_T
\]

\[
\beta_P = -\frac{1}{\rho_0} \left( \frac{\partial \rho}{\partial T} \right)_P
\]  

(10)

called isothermal compressibility, heat capacity at constant pressure and volume thermal expansivity, respectively; we get the following expressions for the fluctuations in entropy, pressure and temperature:

\[
\rho_0 T_0 \delta s = -T_0 m_0 \beta_P \delta P + \rho_0 C_p \delta T
\]

\[
\delta \rho = \rho_0 K_T \delta P - \rho_0 \beta_P \delta T
\]  

(11)

Introducing the equations above in the last equation of Eq. 7 and Eq. 8 results in

\[
\frac{\partial}{\partial t} \left( \rho_0 C_p \delta T - T_0 m_0 \beta_P \delta P \right) = \nabla \cdot [\kappa \nabla (\delta T)] + Q
\]

\[
\frac{\partial^2}{\partial t^2} \left( K_T \delta P - \beta_P \delta T \right) = \frac{1}{\rho_0} \nabla \cdot (\nabla (\delta P))
\]  

(12)
In order to uncouple these equations, it is enough to neglect the thermal conduction term \( \nabla \cdot [\kappa \nabla (\delta T)] \). This assumption can be applied when heat diffusion takes place over a much longer time scale than acoustic propagation, which is the case for water-based liquids under standard conditions. Substituting for \( \delta T \) results in the following reduced wave equation:

\[
\left[ \left( \frac{\rho_0 K T C_P - \beta_p^2 T_0 m_0}{C_P} \right) \frac{\partial^2}{\partial t^2} - \nabla^2 \right] \delta P = \frac{\beta_p}{C_P} \frac{\partial Q}{\partial t} \tag{13}
\]

that can be written in a more suitable form as Eq. 1.

References