MEDICION DE LA VELOCIDAD DE LOS GLOBULOS ROJOS EN MICROVASOS UTILIZANDO LUZ INFRAROJA CERCANA AL ESPECTRO VISIBLE

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Tesis para optar el titulo de maestría en Ingeniería Electrónica

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A mi esposa Juliana, mis Padres , Hermanos y Familia .

A la mujer que inspira mis sueños y mis ilusiones

Juliana …

Measurement of Red Blood Cell Velocity in Micro Vessels Using Near Infrared Light with Cross Correlation Technique.

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ABSTRACT

This document describes the results found measuring blood velocity in micro-vessels using Near Infrared Light (NIR) with Cross Correlation Technique. Measurements made with Infra Red AlGaAs Emitter Diode (IRED) at a peak emission wavelength of 880 nm were compared to measurements performed with a 50 W halogen lamp currently used. The experiments were carried out in 3 male Syrian golden hamsters of 65 ± 5 g body wt. Diameter and velocity were measured to investigate microhemodynamic effects of NIR and halogen light. Tissue in the dorsal skin fold was exposed to NIR irradiance (4.15 mW/cm^2) for up to one hour to test for any NIR related tissue damage. Four arterioles and three venules were selected to acquire the signal average power of the two photodiodes and the corresponding cross correlation signal using six different levels of irradiance for each source of light.

The result shows that when NIR is used the light irradiance on the tissue can be reduced in blood velocity measurements. Usually, the halogen lamp irradiance during the experiments is more than 30 mW/cm^2. Using NIR it is possible to achieve an appropriate cross correlation with an irradiance of 4.15mW/cm^2 which actually is seven times less**.**

The CCD camera used to visualize the microcirculation and to align the photodiodes with the vessels has less saturation using NIR compared with halogen light, thus making it possible to see the blood flow direction and

experiment video recording. This is not possible with halogen light, due to high incident energy in visible spectrum of this light source. In conclusion NIR can be used for as source of light in red blood cell velocity measurements with less irradiance , this result in less damage to the tissue and less interference with the physiological system being monitored .

INTRODUCTION

The cross correlation technique is widely used to determine red blood cell velocity in living specimens by intravital microscopy [1]. The system consists of halogen lamp to transilluminate the tissue, the optics of microscope, a Charge-Coupled Device (CCD) camera to visualize the microvasculature and two photodiodes, which are located in the light path in a distance of 3.2 cm from the CCD sensor. Figure 1. Light emitted by the light source is variably absorbed by tissue as well as by cellular and acellular blood components. Light contrast changes resulting from blood flow are sensed by the two photodiodes aligned in direction of flow using the image generated by the CCD sensor. The cross correlation system is based on the on-line cross-correlation of the delay between optical signatures derived from the photodetectors (photodiodes). The interdetector delay data is computed automatically from the cross-correlation function by a peak detector designed to provide a voltage proportional to the delay to maximum cross-correlation, regardless of intermediate lower peaks.

Most of light sources currently used for this measurement provide light energy almost evenly distributed in the visible spectrum (Halogen Lamp). For identifying and positioning microvessels and the measurement of their diameters a reasonable low irradiance (3 ± 1mW/cm^2) can be used (is sufficient). However, the spectrum emitted by halogen lamps overlaps only poorly with the responsivity of the silicon photodiodes showing a peak at 900

nm. As a consequence, very high irradiance of 30 mW/cm^2 or more usually has to be used for velocity measurements.

Although the sensitivity of the CCD camera sensor peaks at 680 nm, the sensitivity at 900 nm is only 35 % less. Hence the use of NIR light could allow for both the visualization of the microvasculature as well as for the measurement of red blood cell velocity with the cross correlation technique at the same time and with remarkably lower irradiance compared to normal with light.

In recent years, near-infrared light has proven to be useful for the investigation of biological tissues because of the relatively low absorption of water and high absorption of oxy- and deoxyhemoglobin in the near infrared part of the spectrum (700 –1000 nm) [5]. NIR light has been used for the noninvasive spectroscopic determination of oxy- and deoxyhemoglobin concentration in a variety of tissues including muscle, brain and connective tissue [2],[3] and has been shown to transmit not only through muscle but also through skin and bone without high attenuation [4].

Near infrared light can penetrate biological samples with a thickness of 0.5-2 cm, thereby offering the possibility to investigate remote tissues and to differentiate between healthy and diseased tissues.

Rendell et al., have demonstrated that near-infrared spectroscopy can be used to measure also blood flow [5],[6]. Blood has a spectrum quite unlike that of surrounding tissue due to the presence of hemoglobin, and lacks sharply defined peaks.

The aim of the present study was to evaluate NIR as a potential light source for the measurement of red blood cell velocity with cross correlation technique at an irradiance that is less likely to induce tissue damage compared to commonly used halogen lamps. Further on, the measurement of red blood cell velocity under direct visual control as possible with the use of NIR, can be considered to improve the precision of the measurement. The reduction of the irradiance will produce less damage on the tissue, less external factors that affect a non invasive measurement and less energy consumption using a inexpensive source of light.

METHODS

Animal preparation

Experiments were carried out in 3 male Syrian golden hamsters of 65 ± 5 g body wt (Simonsen, Gilroy, CA). All animals were housed in cages with access to food and water ad libitum in a temperature controlled room with a 12:12-h dark-light cycle. Prior to the experiments, dorsal skinfold chambers were implanted and chronic catheters were inserted into the carotid artery and jugular vein of the animals as previously described [7]. Under general anesthesia, the dorsal skin fold consisting of two layers of skin and muscle was fitted with two titanium frames with a 15-mm circular opening and surgically installed. A location that included a paired small artery and vein was selected. Layers of skin muscle were separated from the subcutaneous tissue and removed until a thin monolayer of muscle including the small artery and vein and one layer of intact skin remained. A cover glass (diameter, 12 mm) held by one frame covered the exposed tissue. Polyethylene tubes (PE-10, 1 cm; Becton Dickinson, Parsippany, NJ) were connected to a PE-50 tube (25 cm) via silicone elastomer medical tubes (4 cm, Technical Products) and were implanted in the jugular vein and the carotid artery. They were passed from the ventral to the dorsal side of the neck and exteriorized through the skin at the base of the chamber. Microvascular observations in the awake hamsters were performed at least 2

days after chamber implantation to exclude the influence of surgical trauma and anesthesia. During the measurements the animals were placed in a perforated plastic tube (inner diameter, 3.8 cm; length, 17 cm), from which the window chamber protrudes, to minimize animal movement without impeding respiration. A preparation was considered suitable for experimentation if it was void of edema, inflammation and bleeding.

Instrumentation

Microvessels in the subcutaneous tissue and the striated skin muscle were observed with an inverted microscope (IMT-2, Olympus, Tokyo, Japan) using a x20 objective (Olympus). Microscopic images were obtained by a CCD camera (Cohu 4815–2000, San Diego, CA), displayed (Sony Trinitron PVM-1271Q monitor, Tokyo, Japan) and transferred to a videorecorder (AG-7355; Panasonic, Tokyo, Japan). The arteriolar and venular segments under study were selected for their optical clarity. Vessel diameter was measured with an image-shearing system (Digital Video Image Shearing Monitor 908, IPM, San Diego, CA), whereas RBC velocity was analyzed by photodiodes and the cross-correlation technique (Velocity Tracker Mod-102 B, IPM) [2]. The experiment setup is show in figure 1.

Source of light

An AlGaAs Infrared Emitter Diode (IRED) with Peak Emission Wavelength of 880 nm, encapsulated in a clear, peach tinted, plastic TO-46 package (QUD123, Fairchild semiconductor, Irving, TX) was used as a source of near infrared light. The LED was positioned in a distance of 14 cm above the skinfold chamber and connected to a power supply regulated at 0 to 30 V. A 50W halogen lamp (OSRAM) was used for the normal velocity measurements. Orthogonal light to the preparation passed a condenser, the

position of which was adapted for each light source in order to keep the irradiated tissue surface constant at 1.77 mm^2.

Irradiance Measurement

Irradiance is the measurement of the density of light striking a surface. The most conventional units of measurement are Watts per square centimeter (W/cm^2) [3]. The Irradiance was measured with Radiometer/Photometer (1400A, International Light, Inc, Newburyport, MA) with the sensor SEL033 base detectors. Before the experiments, the irradiance on the surface of the skinfold chamber was measured with a constant distance between the microscope condenser and the hamster preparation for each light source.

Infrared radiation

Before the performance of the NIR in velocity measurements was evaluated, the whole circumference of the skinfold chamber was exposed to NIR with power of 4.15mW/cm^2 for 60 minutes in a separate animal to investigate potentially harmful effects of NIR on tissue integrity and microvascular parameters. Velocity and diameter measurements were made in identical arteriolar and venular vessel segments before and immediately after the exposure (60 min) (Figure 2) (A). The NIR exposure was recorded and photographs were taken before, immediately after, and 24 hours after exposure.

Velocity measurement with NIR and halogen lamp

In separate animals, velocity, signal average power of the photodiodes and the cross correlation signal from the velocity instrument were assessed. Four arterioles and three venules were selected and classified by diameter. For the comparison of NIR with normal white light, paired velocity measurements were performed in identical vessel segments, first with halogen light then with NIR light. Velocity was determined at the highest level of irradiance; subsequent measurements were taken after decrementing the irradiance. Once the cross-correlation was found for each level of irradiance three signals were acquired (Signal photodiode A, Signal photodiode B, Cross Correlation curve). nine levels of irradiance were selected for the halogen lamp ranging: [0.6, 1.1, 1.56, 2.55, 4.15 , 5.8 , 10.1 , 17.5 , 29.2] mW/cm^2 and eight levels for NIR from [0.53, 0.75, 1.28, 1.85, 2.34, 2.92, 3.53, 4.15] mW/cm^2, which was the maximum irradiance achievable with this specific type of IRED.

In one of the arterioles, the saturation of the CCD camera was tested with each light source. Video frames were taken for different irradiance levels and were compared to the corresponding cross correlation response from the velocity machine (see figure 6 and figure 7). For both light sources, a gray level histogram was established for those images that were recorded at the respective peak cross correlation. The histogram shows the density of gray levels classified in 255 points from black color (0) to white color (255).

RESULTS

Infrared radiation

The results showed that NIR irradiation with a power of 4.15 mW/cm^2 for one hour does not cause vasoconstriction and decrease of blood flow. Even after this prolonged exposure with maximum intensity, the changes observed were limited to an increase of the diameter of 8.4 % in arterioles and of 7.9 % in venules (figure 3), accompanied by a decrease of velocity less than 23 % in arterioles and 30% in venules (figure 4). However, the photos taken before and after the exposure showed no signs of impaired tissue integrity (i.e. bleeding, inflammation, edema) (figure 2B).

Velocity measurement with NIR and halogen lamp

Four arteries and three veins were selected to determine velocity, the signal average power of the photodiodes and the cross-correlation with NIR and white light. The results of the velocity measurement obtained with NIR did not differ significantly from the values measured with the use of the halogen lamp (figure 5). The mean difference between the measurements was 8% for the arterioles and 25% for the venules.

For both NIR and white light the peak correlation was found at the highest irradiance of the respective light source. The maximum NIR irradiance value was set to 4.15mW/cm^2. At this intensity, the correlation peak found with NIR

was higher in arterioles (53%) and venules (44.5 %) than the respective values found with the use of white light. In arterioles the correlation peak was 1.3 ± 0.05 V with NIR compared to 0.61±0.25V with visible light. In venules the correlation peak was 1.1 ± 0.25 V with NIR compared to 0.61±0.3V with visible light (figure 8 and 9).

The average power of the photodiode signals for arterioles was 47.7 % higher for NIR compared to the halogen lamp. For venules the difference was 17.7%.

The gray level histogram for the halogen lamp (Figure 12.A) shows a high number of white pixels (250-255), only 10% of pixels were found in the lower grey levels (200 to 250). As a consequence, most of the visible information contained in the image was lost when the peak correlation was reached. With NIR the histogram shows an increment of 300 % in the lower gray levels between 50 to 200, in others words characteristics of the image (fat cells and vessel of interest) can be observed (figure 12.B). In a normal experiment this factor is very important since artifacts due the movement of the animals can only be avoided when there is a chance to detect those and to re-align the photodiodes. Is possible that the user made an error in the velocity measurement value with the cross correlation technique because when the CCD camera is saturated the user can not see the movements of the animal and an incorrect velocity value could be acquired.

DISCUSSION

The infrared radiation that is possible to use NIR to measure velocity without damage to the tissue even after one hour of near infrared light exposure. This exposure tested is ten times more than a normal velocity measurement. This result allow us to perform velocity measurements with NIR without causing damaging effects at the irradiance level of 4.15 mW/cm^2 and peak wave length of 880 nm .

It was found that the use of 880 nm NIR optimized the performance of the sensor in the cross correlation technique with silicon photodiodes, although the specific absorption coefficient of the HbO_2 and HbCO in the near infrared light is 10^2 times less than the visible light [9]. The cross correlation response shows a peak of 1.3 ± 0.35 for NIR at the maximum level of irradiance 4.15mW/cm^2 and velocity values which are into the range of the red blood cell velocity [7].

The average power of the photodiode signal in arterioles with NIR at only 4.15mW/cm^2 was 47.7% higher than the halogen lamp at the same level of irradiance. These results confirm that the energy incident on the tissue can be reduce in order to diminish the tissue damage whit change the average power of the photodiodes.

Advantages were found in the image used to re-align the photodiodes. This advantage consists

In order to the image captured with NIR was not saturated in comparison with halogen lamp and at the same time had a good peak of cross correlation of 1.35 V from the velocity cross correlation. The time required for aligning the sensor and also light exposure time is reduced due to the ability to see the vessel and the blood flow in the image from CCD camera.

In this laboratory we have developed a lamp that can be placed above the microscope condenser and generate the irradiance levels for the velocity measurements using NIR. An option has been left open with a source of light in three different wave lengths in the visible spectrum. This experiment could be interesting for obtainig velocity measurements and combining different irradiance levels with NIR . This configuration can be optimized to reduce tissue damage and optain high absorbtion from HbO_2 , while using a low constant irradiance level to measure both vessel diameter and blood velocity.

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visible and near infra-red regions, *J. Biol. Chem.*, **148**, pp173-183.

FIGURES

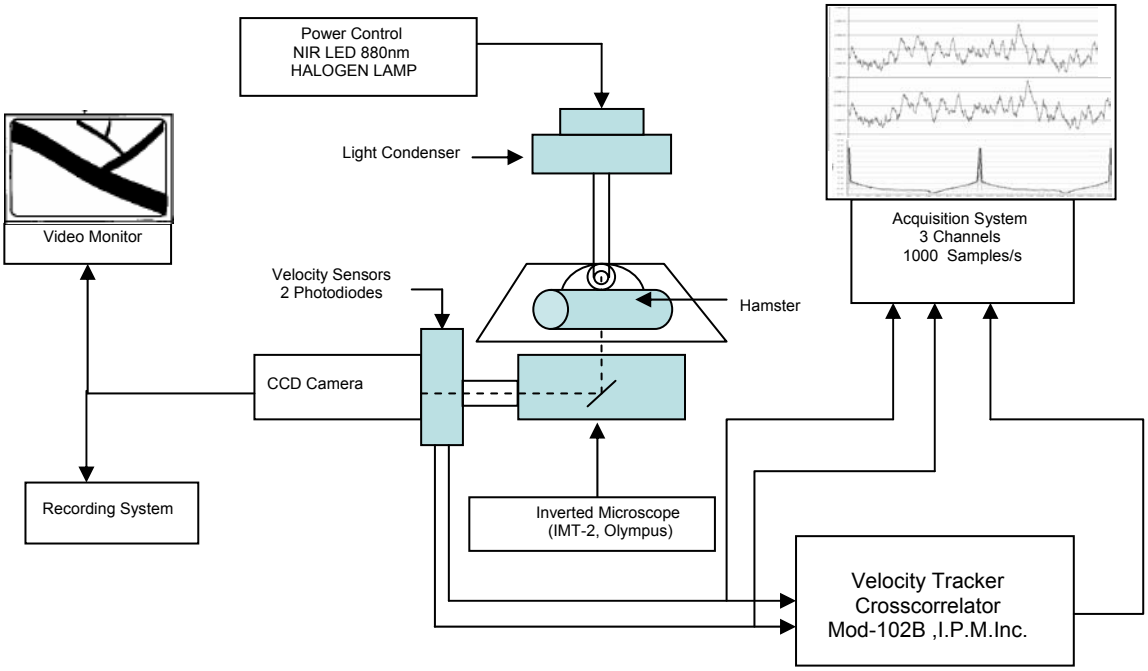


Fig. 1. Schematic diagram of the experimental setup. A personal computer with Analog to digital converter was used to acquire the three signals for the analysis of signal average power and delay correlation.

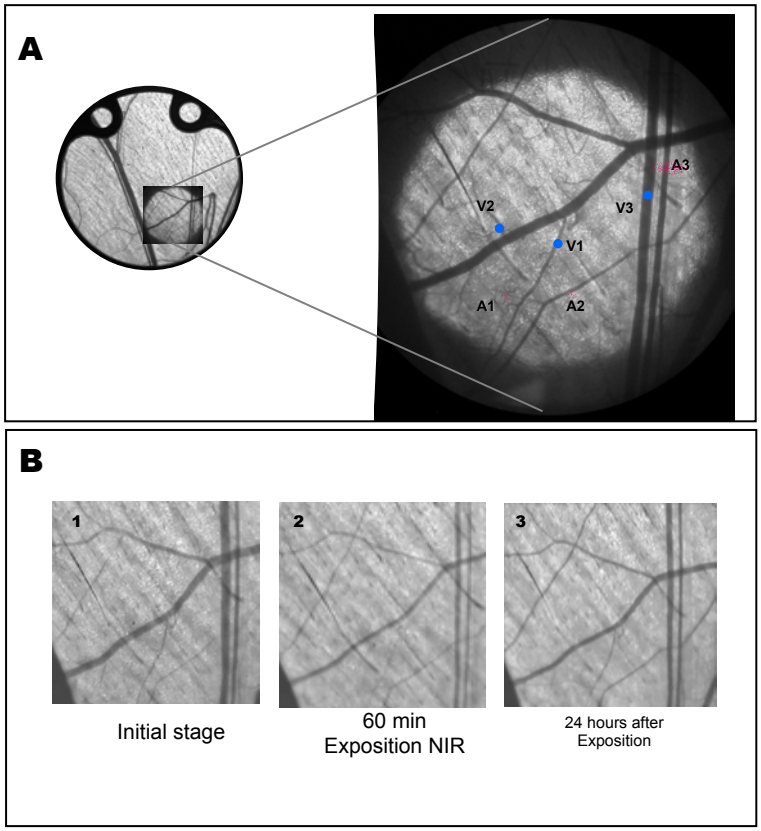
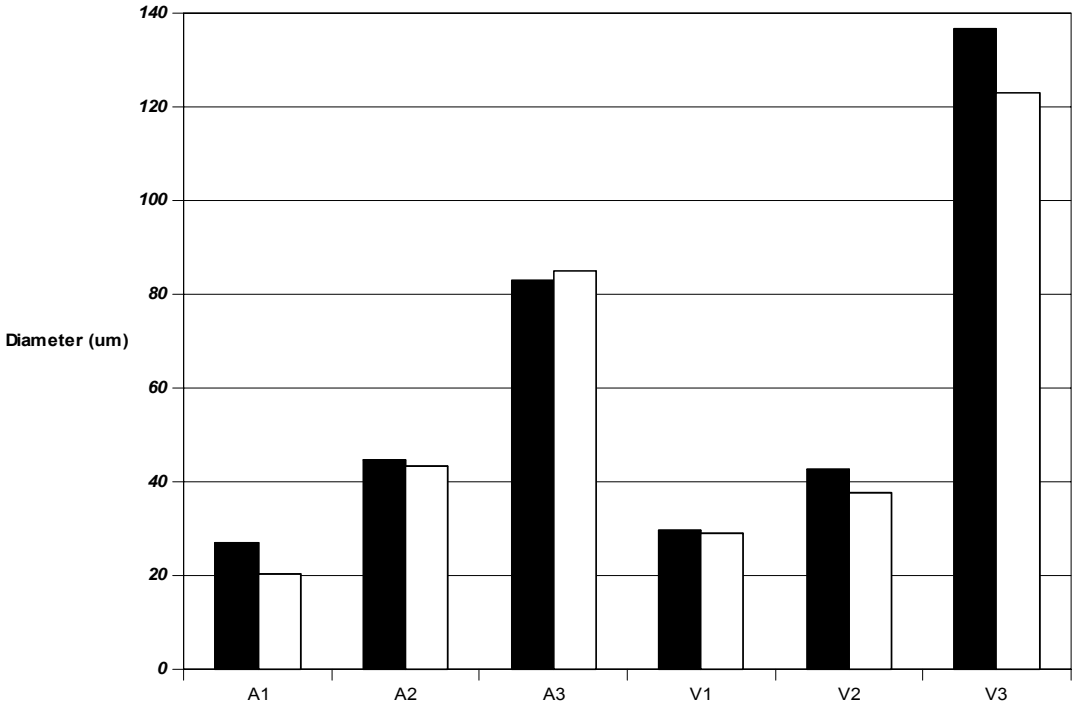
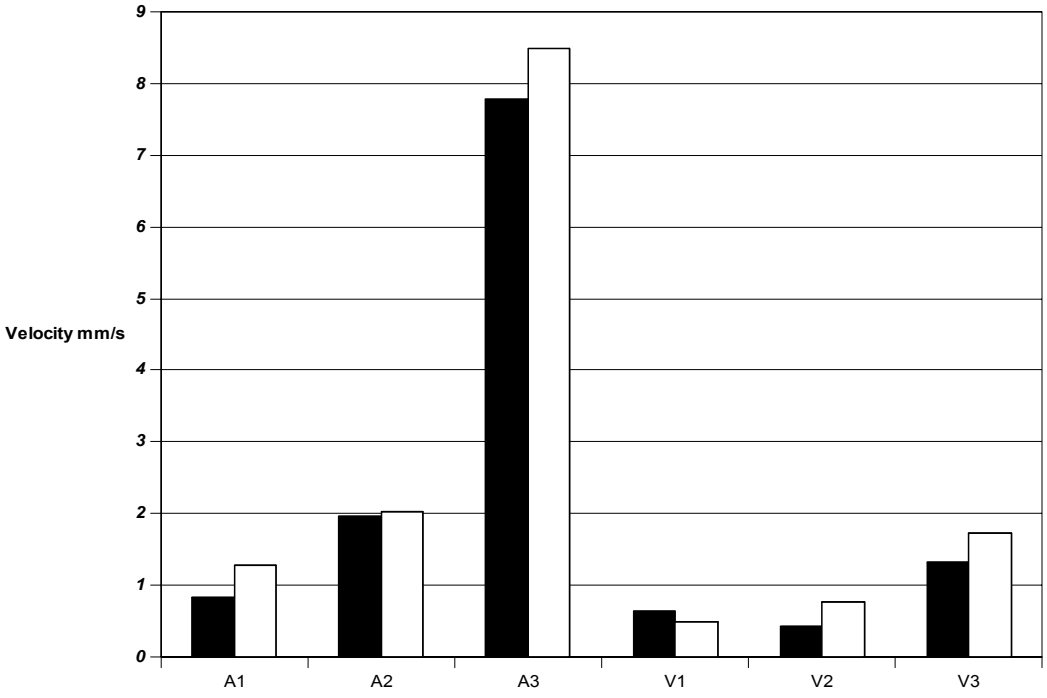


Fig. 2. Chamber area used for NIR irradiation. A) Veins and arteries selected. B) Photos of the tissue before and after NIR exposure.



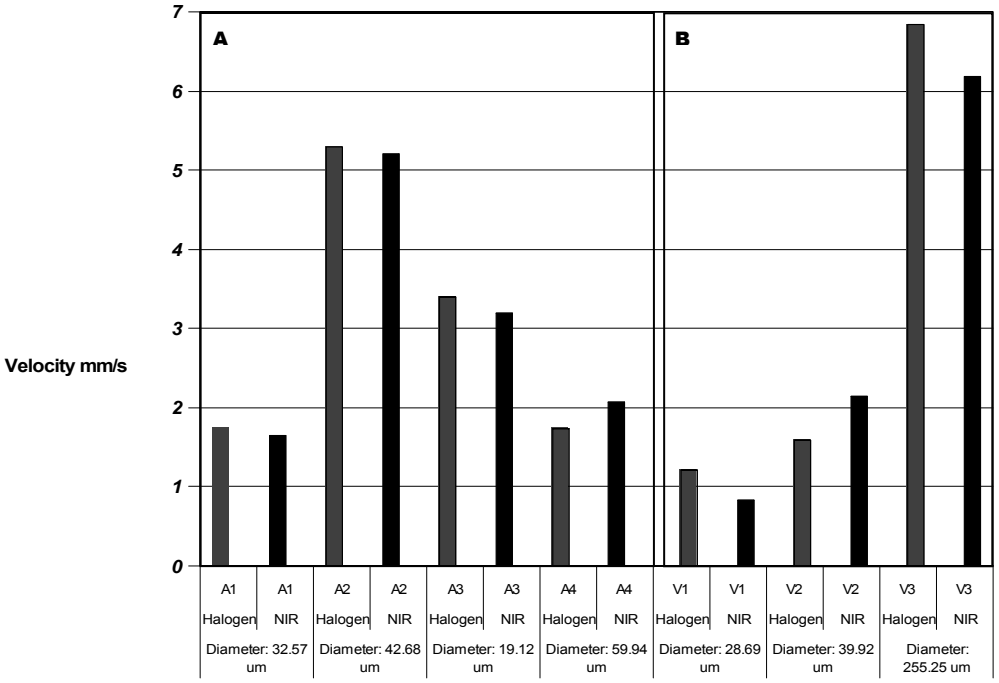
	A1	A2	A3	V1	V2	V3
■ diameter t=0	27.04	44.6	82.95	29.51	42.6	136.65
□ diameter t= 1Hour	20.28	43.32	85.01	28.88	37.6	122.97

Fig. 3. Diameter of the arteries and veins before and after of 60 minutes of Near Infrared Light exposition. Irradiance 880 nm 4.15 mW/cm^2.



	A1	A2	A3	V1	V2	V3
■ Velocity t=0	0.8225	1.96	7.8	0.636	0.4074	1.32
□ Velocity t= 1Hour	1.28	2.03	8.5	0.48	0.75	1.73

Fig. 4. Velocity of the arteries and veins before and after of 60 minutes of Near Infrared Light exposition. Irradiance of 880 nm 4.15 mW/cm^2



	Diameter: 32.57 um		*Diameter: 42.68 um*		*Diameter: 19.12 um*		*Diameter: 59.94 um*		*Diameter: 28.69 um*		*Diameter: 39.92 um*		*Diameter: 255.25 um*	
	Halogen	*NIR*	*Halogen*	*NIR*	*Halogen*	*NIR*	*Halogen*	*NIR*	*Halogen*	*NIR*	*Halogen*	*NIR*	*Halogen*	*NIR*
	A1	*A1*	*A2*	*A2*	*A3*	*A3*	*A4*	*A4*	*V1*	*V1*	*V2*	*V2*	*V3*	*V3*
■ *velocity of the Arteries*	*1.73*	*1.65*	*5.3*	*5.2*	*3.4*	*3.2*	*1.73*	*2.07*						
□ *velocity of the Veins*									*1.207*	*0.83*	*1.5882*	*2.1408*	*6.84*	*6.19*

Fig. 5. Velocity measured with two different sources of light ; Halogen Lamp and Near Infrared Light. A) Arteries B) Veins

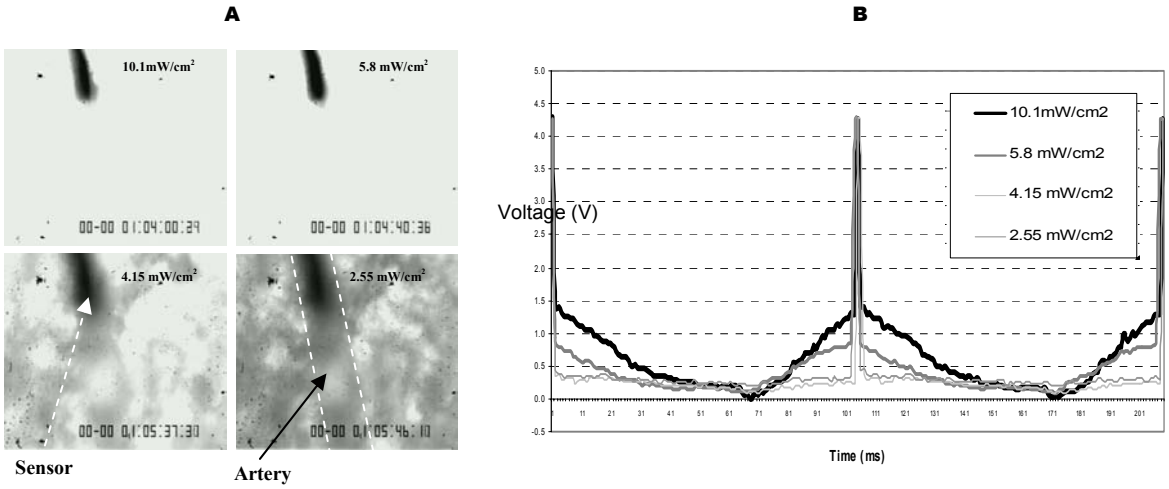


Fig. 6. Comparison between correlation peak and the corresponding image captured with the camera using ***halogen lamp***. A) Image obtained for each irradiance. B) Correlation signal for each irradiance .

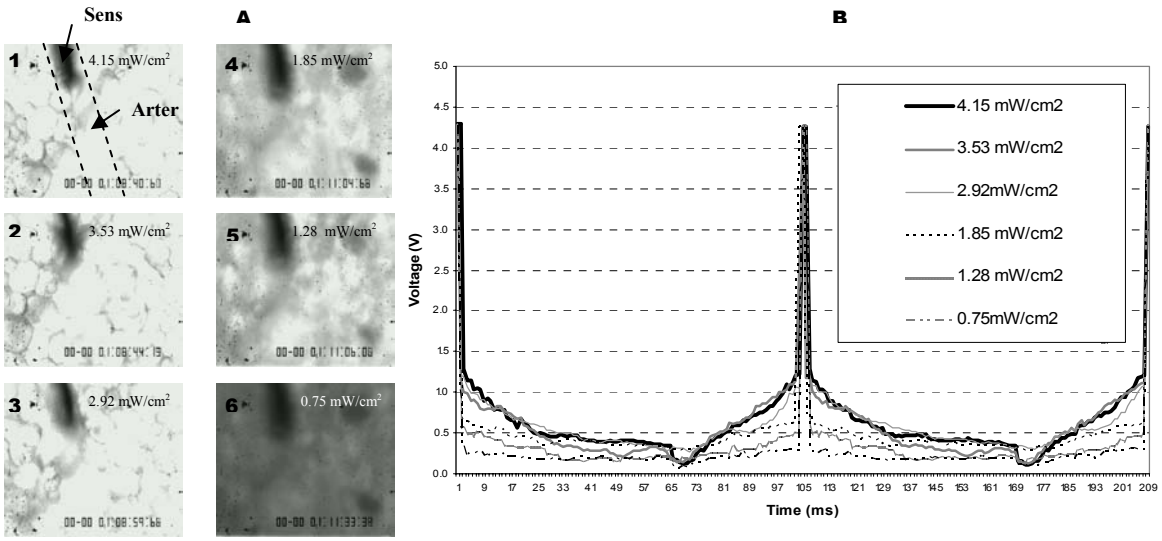


Fig. 7. Comparison between correlation peak and the corresponding image captured with the camera using ***NIR 880 nm***. A) Image obtained for each irradiance. B) Correlation signal for each irradiance .

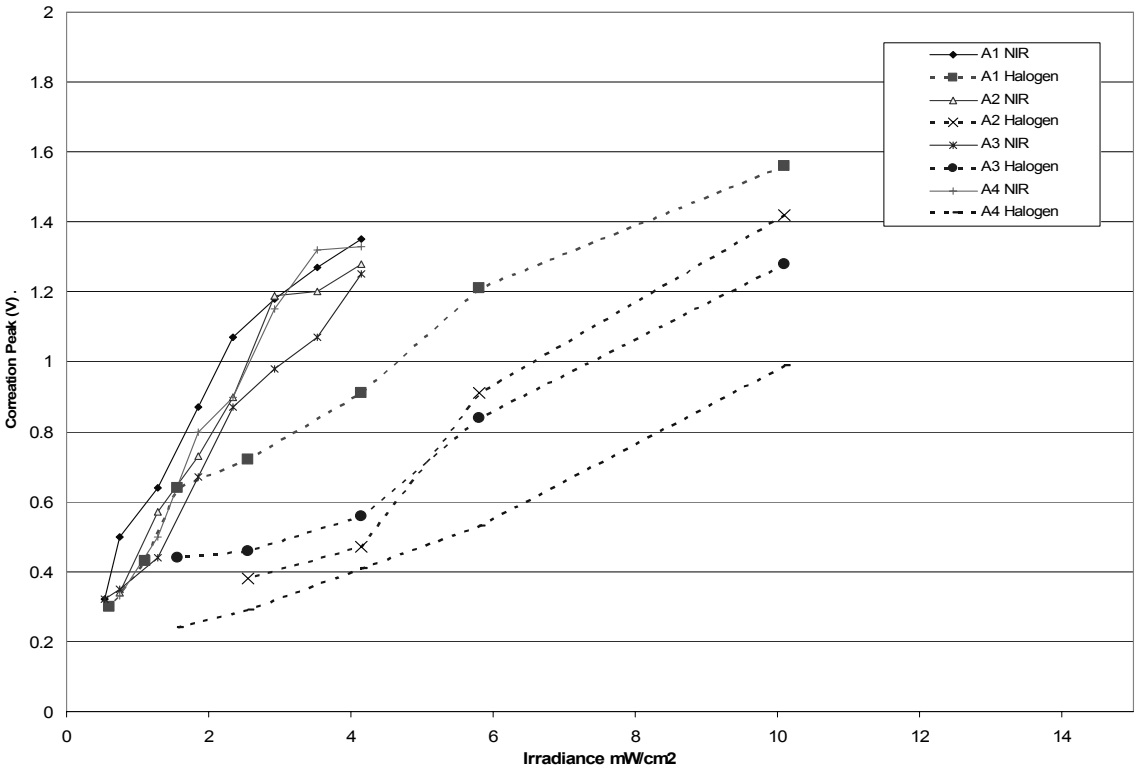


Fig. 8. Magnitude of the cross correlation response with different irradiance for NIR and Halogen lamp in arteries.

The level obtained for NIR shows that is possible to obtain the velocity with high correlation peak using less irradiance (4.15 mW/cm^2)

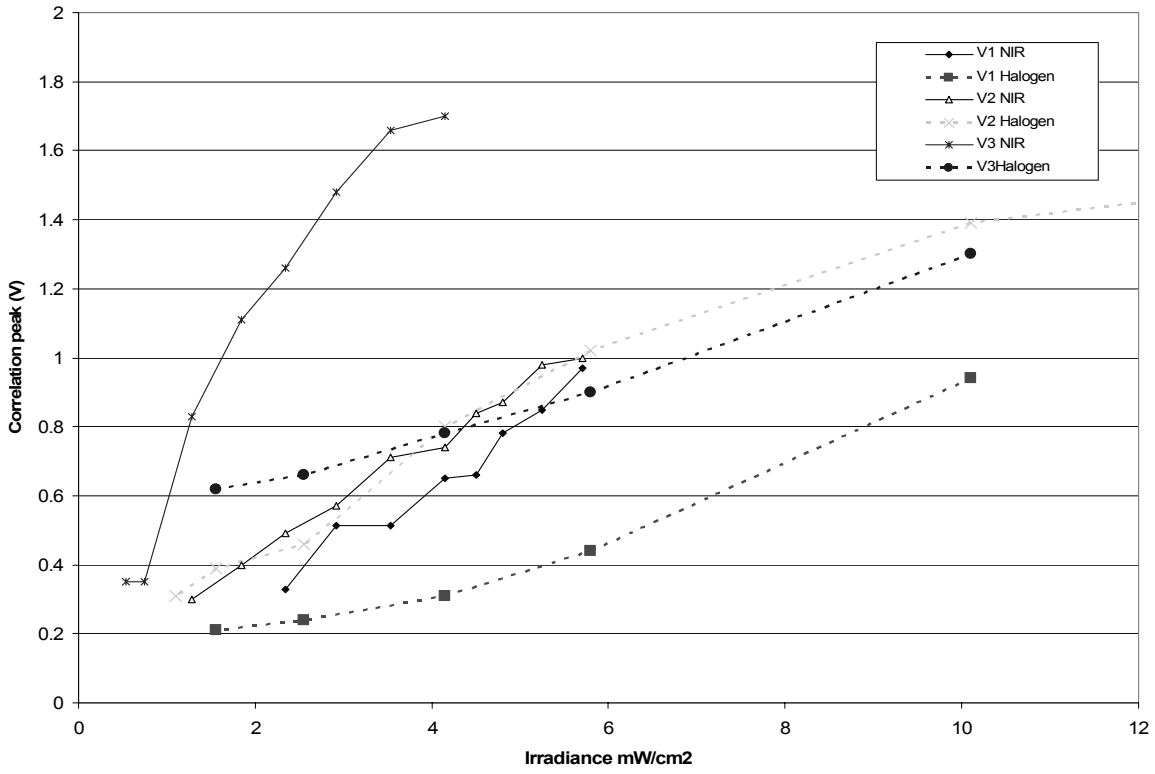


Fig. 9. Magnitude of the cross correlation response with different irradiance for NIR and Halogen lamp in veins .
The level obtained for NIR shows higher values at (4.15 mW/cm2) irradiance than halogen lamp.

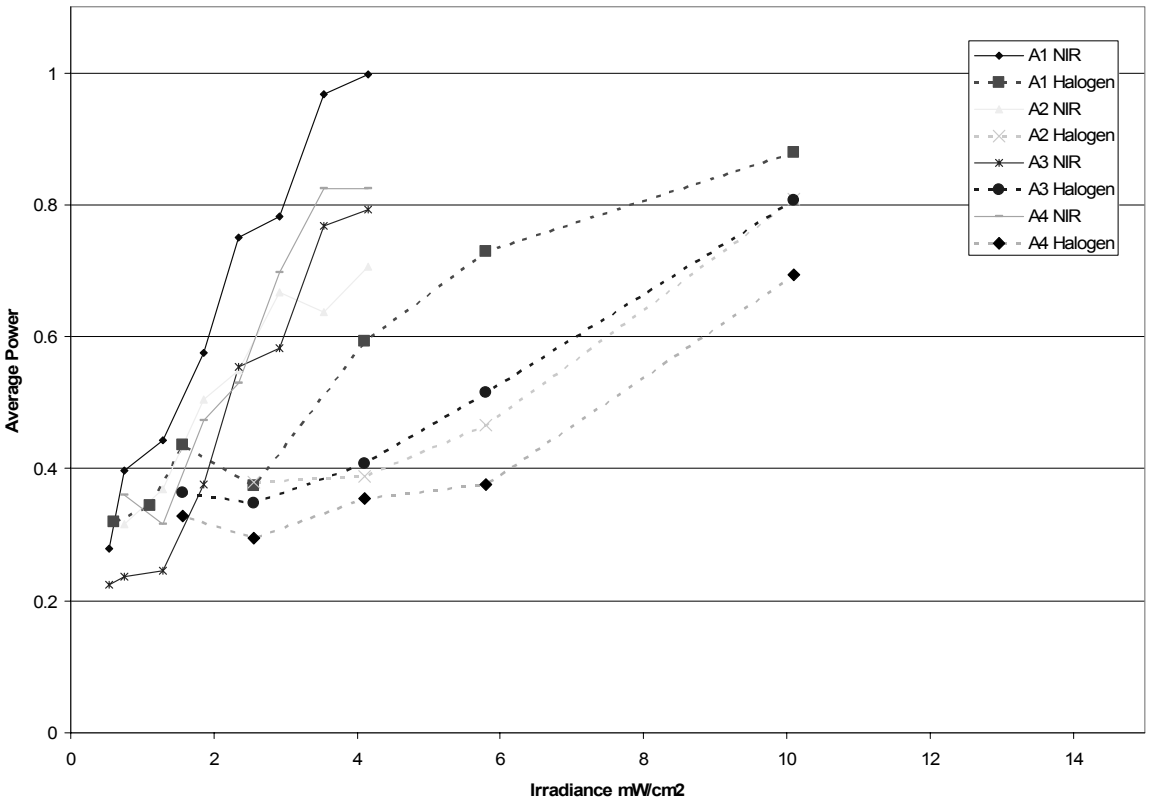


Fig. 10. Average power of the signals obtained from the velocity sensor (silicon photodiode) in ***Arteries***. It is possible to register the same average power with NIR using one third as much irradiance than halogen lamp irradiance.

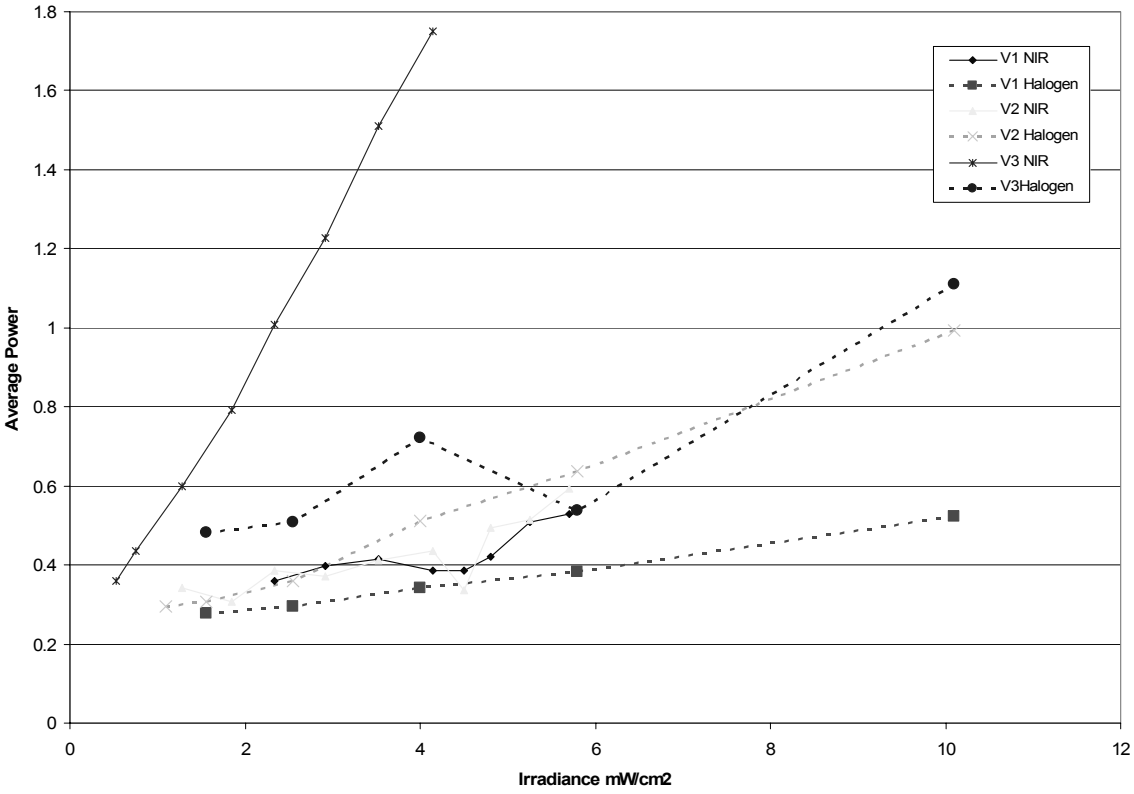


Fig. 11. Average power of the signals obtained from the velocity sensor (silicon photodiode) in ***veins***. The NIR presents a higher average power in comparison to the halogen lamp.

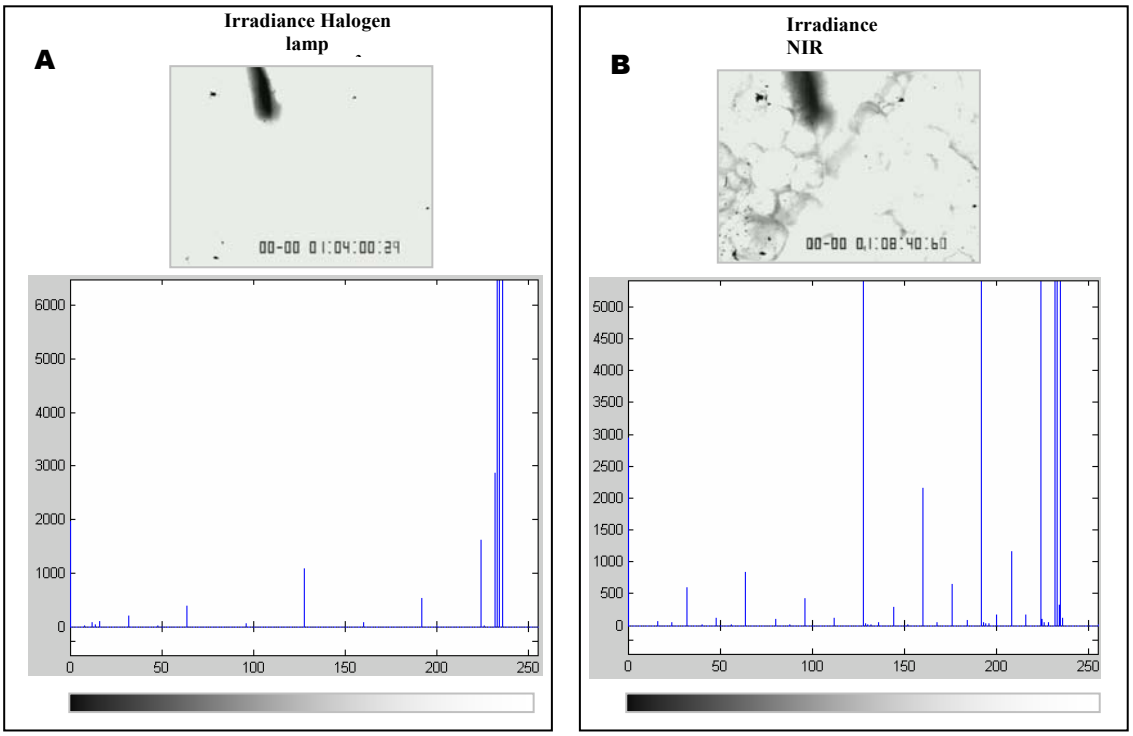


Fig. 12. Histogram of the images at the 1.35 V level of the peak in cross correlation response . A) Image and histogram for the halogen lamp irradiance . B) Image and histogram for the NIR . The NIR image shows the fat cells and the artery in the velocity measurement without saturate the image , in contrast with halogen lamp that need of high energy to find cross correlation with the photodiode sensors.

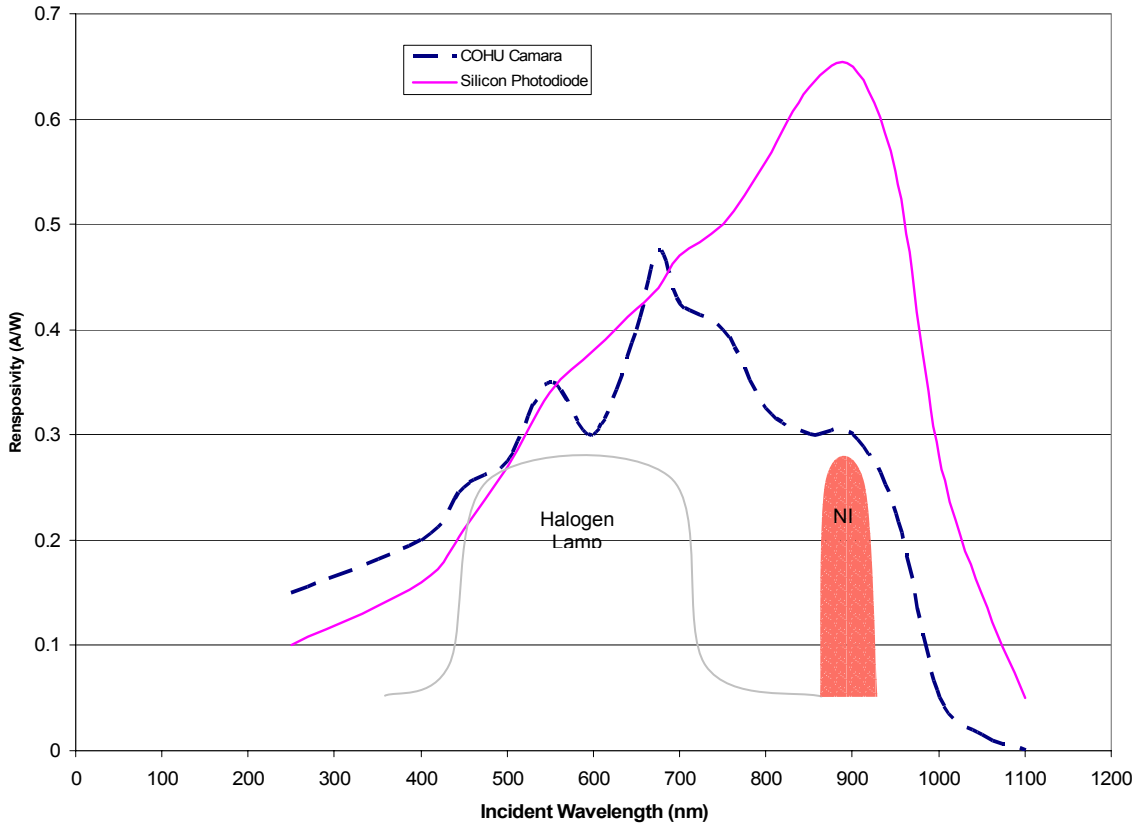


Fig. 13. Responsivity of the CCD camera and silicon photodiode. Spectral response for the two source of lights used during the experiments. Halogen Lamp and Near infrared light .