Tesis de lic

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Resumen

Índice general

Capítulo 1

Introducción

1. que es la luminiscencia? algunas aplicaciones ej. microscopía, plim y flim

    - In 1565, a Spanish physician and botanist, Nicolas Monardes (Figure 2), reported the peculiar blue color (under certain conditions of observation) from an infusion of a wood from Mexico and used to treat kidney and urinary diseases (Figure 3). 912 This wood (later called Lignum nephriticum), whose peculiar color effect and diuretic properties were already known to the Aztecs, was a scarce and expensive medicine. Therefore, it was of interest to detect counterfeited wood. Monardes wrote on this respect, 12 Make sure that the wood renders water bluish, otherwise it is a falsification. Indeed, they now bring another kind of wood that renders the water yellow, but it is not good, only the kind that renders the water bluish is genuine. (in Spanish in the original). This method for the detection of a counterfeited object can be considered as the first application of the phenomenon that would be later called fluorescence. Extracts of the wood were further investigated by Boyle, Newton, and others, 6 but the phenomen- on was not understood at the time.
    - n 1845, the polymath Sir John Herschel, son of the famous astronomer and the originator of the word photography (where light is again involved), prepared an acid solution of quinine sulfate and stated,18 Though perfectly transparent and colorless when held between the eye and the light, it yet exhibits in certain aspects, and under certain incidences of the light, an extremely vivid and beautiful celestial blue color. As the color was always superficial, he believed it to be a hitherto unidentified phenomenon, “a case of superficial colour presented by a homogeneous liquid, internally colourless”. 18 Herschel called this phenomenon epipolic dispersion, from the Greek: = surface. In fact, the solutions observed by Herschel were very concentrated so that the majority of the incident light was absorbed near the surface and all the blue fluorescence originated from there. Herschel used a prism to show that the epipolic dispersion could be observed only upon illumination by the blue end of the spectrum and not the red end. The crude spectral analysis of the emitted light with the prism revealed blue, green, and a small quantity

of yellow light, but Herschel did not realize that the superficial light was of longer wavelength than the incident light.

- One of Stokes's experiments that is spectacular and remarkable by its simplicity deserves attention. Stokes formed the solar spectrum by means of a prism. When he moved a test tube filled with a solution of quinine through the visible part of the spectrum, nothing happened: the solution remained transparent. 20 But beyond the violet portion of the spectrum, that is, in the invisible zone corresponding to ultraviolet radiation, the solution glowed with a blue light (Figure 7). Stokes wrote, 4 It was certainly a curious sight to see the tube instantaneously light up when plunged into the invisible rays: it was literally darkness visible. Altogether the phenomenon had something of an unearthly appearance. From his experiments with a wide range of substances, Stokes concluded that the dispersed light was always of longer wave- lengths than the incident light. Later this statement became the Stokes law. Stokes also noted that the dispersion of light took place in all directions, hence, the fluid behaved as if it were self-luminous. In his paper, Stokes called the observed phenomenon true internal dispersion or dispersive reflection but in a footnote, 4 he wrote, I confess I do not like this term. I am almost inclined to coin a word, and call the appearance fluorescence, from fluorspar, as the analogous term opalescence is derived from the name of a mineral. In his second paper, 21 Stokes definitely resolved to use the word fluorescence.
- Luminescence is an emission of ultraviolet, visible or infrared photons from an electronically excited species. The word luminescence, which comes from the Latin (lumen ¼ light) was first introduced as luminescenz by the physicist and science historian Eilhardt Wiedemann in 1888, to describe 'all those phenomena of light which are not solely conditioned by the rise in temperature', as opposed to incan- descence. Luminescence is cold light whereas incandescence is hot light. The various types of luminescence are classified according to the mode of excitation (see Table 1.1).
- The success of fluorescence as an investigative tool in studying the structure and dynamics of matter or living systems arises from the high sensitivity of fluoro- metric techniques, the specificity of fluorescence characteristics due to the micro- environment of the emitting molecule, and the ability of the latter to provide spatial and temporal information. Figure 1.3 shows the physical and chemical parameters that characterize the microenvironment and can thus affect the fluorescence char- acteristics of a molecule.
- As a consequence of the strong influence of the surrounding medium on fluores- cence emission, fluorescent molecules are currently used as probes for the investi- gation of physicochemical, biochemical and biological systems. A large part of this book is devoted to the use of so-called fluorescent probes.
- An electronic transition consists of the promotion of an electron from

an orbital of a molecule in the ground state to an unoccupied orbital by absorption of a photon. The molecule is then said to be in an excited state. Let us recall first the various types of molecular orbitals.

- Additionally, fluorescence is used for cell identification and sorting in flow cytometry, and in cellular imaging to reveal the localization and movement of intracellular substances by means of fluores- cence microscopy.
- It is interesting to notice that the first known fluoro- phore, quinine, was responsible for stimulating the devel- opment of the first spectrofluorometers, which appeared in the 1950s. During World War II, the Department of De- fense was interested in monitoring antimalaria drugs, in- cluding quinine. This early drug assay resulted in a subsequent program at the National Institutes of Health to develop the first practical spectrofluorometer.*

2. hamiltoniano de un átomo de muchos electrones? - si hago esto, el foco debería ser
   por qué las cosas emiten o no emiten?
3. diagrama de jablonski. Cómo se convierte la excitación del electrón en luz? de qué otras formas puede convertirse la energía del electrón? decaimientos radiativos y no radiativos.
   ver seccion jablonski del lakowicz
4. fluorescencia y fosforescencia diferencias ->transiciones prohibidas y tiempos de vida
5. corrimiento stokes y anti stokes ->fenómenos de muchos fotones
6. caracterización de luminiscencia estática
7. caracterización de luminiscencia dinámica ->TCSPC
8. Nanopartículas de upconversion, composición y aplicaciones
9. fotofísica de los lantánidos
10. mecanismos principales de upconversión
11. dependencia con la potencia de excitación
12. modelos de la dinámica de las nanopartículas
13. que hace falta para caracterizar las nanopartículas?

agregar fenómenos de fosforescencia más antiguos En 1565, el médico y botanista Nicolás Monardes reportó el peculiar color azul que tomaba una infusión de madera mexicana usada para tratar enfermedades de riñón y urinarias. Este efecto ya era conocido por los Aztecas, que lo utilizaban para asegurarse que la valiosa madera no fuera falsificada. Monardes escribe en su libro [**?**]

> *Asegúrate de que la madera torne el agua azulada, de lo contrario, es una falsificación. De hecho, ahora traen otro tipo de madera que torna el agua amarilla, pero no sirve; solo el tipo que torna el agua azulada es genuina.*

Años más tarde, en 1845, el matemático Sir John Herschel describió el efecto similar que producía una solución transparente de quinina, una sustancia presente en el agua tónica, que reflejaba «un color azul celestial hermoso y extremadamente vívido». Herschel usó un prisma para comprobar que la dispersión causada por la quinina sólo se observaba al iluminar la solución con la parte azul del espectro. El mismo análisis para la luz emitida reveló luz azul, verde, y una pequeña cantidad de amarillo. En esa misma época, el físico Sir George Gabriel Stokes publicó *On the Refrangibility of Light*, un trabajo explicando experimentos con múltiples sustancias que exhibían este tipo de comportamientos, entre ellas incluida la quinina. Uno de sus experimentos más importantes consistía en formar el espectro solar a partir de un prisma, para luego mover un tubo de ensayo con la solución de quinina a través de sus colores. La solución permanecía transparente al ser iluminada por la parte visible del espectro, pero al llegar a la zona ultravioleta (invisible al ojo humano), la muestra se iluminó con luz azul brillante. Además de concluir que la luz siempre se dispersaba con longitudes de onda mayores a las de incidencia, afirmación que luego se conocería como corrimiento de Stokes, llamó a este fenómeno *fluorescencia* [**?**].

En 1888, el físico Eilhardt Wiedmann introdujo el término luminiscencia para referirse a los fenómenos lumínicos que no están determinados por un aumento en la temperatura de los materiales. El desarrollo de la mecánica cuántica durante el siglo 20 dió a conocer el fenómeno detrás de la emisión de luz sin aumento de temperatura: las transición de los electrones entre los distintos niveles de energía de un átomo.

Entre ellos está la fosforescencia introducir ejemplo que mencioné antes y de fluorescencia como el de la quinina descripto por Stokes. Actualmente, tanto la fluorescencia como la fosforescencia tienen aplicaciones en múltiples areas distintas del conocimiento y la tecnología. Es particularmente destacable su éxito como herramienta para estudiar la estructura y dinámica de la materia viva, gracias la sensibilidad al micro-entorno de las moléculas fluorescentes, lo que resulta en una alta resolución espacial y temporal. Por ejemplo, la microscopía de fluorescencia consiste en iluminar la muestra con una longitud de onda y detectar su fluorescencia en otra, permitiendo filtrar el fondo de la imagen [**?**]. Técnicas dinámicas como la microscopía de imágenes de tiempo de vida de fluorescencia (FLIM) o fosforescencia (PLIM) dan lugar a conocer el entorno químico en el que se encuentran distintas proteinas [CITA].

1.1. Espectrofotometría estática

1.2. Espectrofotometría dinámica

1.3. Nanopartículas de conversión ascendente

Capítulo 2

Espectrofluorímetro para la caracterización de UCNPs

2.1. Instrumentación para espectrometría de fluorescencia

Para caracterizar la respuesta óptica de una sustancia, Generally, one wishes to record both excitation and emission spectra. the go-to scientific instrument for this measurements is an spectrofluorimeter

2.1.1. ¿Qué es un espectrofluorímetro? (cap 2 lako)

A spectrofluorometer enables steady-state intensity measurements such as wavelength scans, time-based ex- periments, synchronous scans, and polarization of luminescence materials aiding research across various sci-entific disciplines including chemistry, biochemistry, pharmacology, environmental science, materials science,and biomedical research. The goal of a spectrometer is to get the emission and excitation spectrums of a sample. For that it has to be able to illuminate a sample with multiple different wavelengths, and record the sample reaction for multiple different wavelengths too.

Figure 2.1 shows a schematic diagram of a general-purpose spectrofluorometer. It shows all key components to fulfill its purpose Such lamps are generally useful because of their high intensity at all wavelengths ranging upward from 250 nm. The instrument shown is equippedwith monochromators to select both the excitation and emission wavelengths. monochromators are usually motorized to allow automatic scanning of wavelength. . This selected excitation light is then focused onto the sampl The sample luminescence, which usually has a longer wavelength than the excitation light, is then filtered by the emission monochromator. The light left then arrives at a detector, usally a photomuyltiplier tube PMT, which is a sensitive detector that converts photons into an electric current spectrofluorometers and all its components use different techniques to decrease stray light light of wavelengths different from the chosen wavelength), like 90degree angle from excitation and emission arm, sealed tight box covered with non-reflective black paint the pmt signal is then usually processed by electronics, and then digitized and ananlyzed with a PC. This PC also

orchestrates the monochromators and the acquisition, while providing the user a way to adjust parameters of interest and facilitate data visualization and analysis usually, aditional components are added in the light path to study different properties of the sample, like shutters, polarizers, beam splitters and other optics elements.

1. que mide un espectrofluorimetro?
2. cuales son sus componentes?
3. que variables puede controlar?

2.1.2. Epectrofluirimetros en argentina y obsolescencia

espectrofluorimetros disponibles para hacer exp en exactas - sus problemas.

problema no particular de espectrofluorimetria. instrumentos componentes centrales de la investigación científica. obsolescencia de instrumentos: se hecha a perder financiamiento y no se pueden estudiar areas. Intervenir instrumentos closed source para mejorarlos y open source.

alternativa open source para instrumentación. Herramientas open source en general para hacer ciencia e instrumentación.

esta parte de la tesis explica la renovación del horibapti quantamster400

2.1.3. Espectrofluorímetro HoribaPTI QuantaMaster 400

serie horiba quantamaster. frecuencia de aparición en exactas y arg en general, baratos, etc. quizás mencionar lo de stefani

componentes de funcionamiento: conectores originales, lampara, monocromadores, chamber, pmt, especificaciones

software de control: felix gx, capacidades fundamentales y deficiencias

que falta para caracterizar ucnps? time-consuming experiments, operación, medición de tiempos de vida, etc.

2.2. Renovación de HoribaPTI QuantaMaster 400

2.2.1. Hardware quizás es al pedo diferenciar entre hw y sw

Qué reemplazamos y con qué nos quedasmos, especificaciones finales

2.2.2. Software

capacidades del software

Jerarquía de clases API para desacoplar. Extensión del software: sirve para espectrofluorímetro genérico siempre y cuando tengas motor por pasos y pmt.

quien cuenta los picos, (mencionar que hay analisis de picos más adelante)

Modos de uso, API GUI (capacidades, más detallado en apéndice).

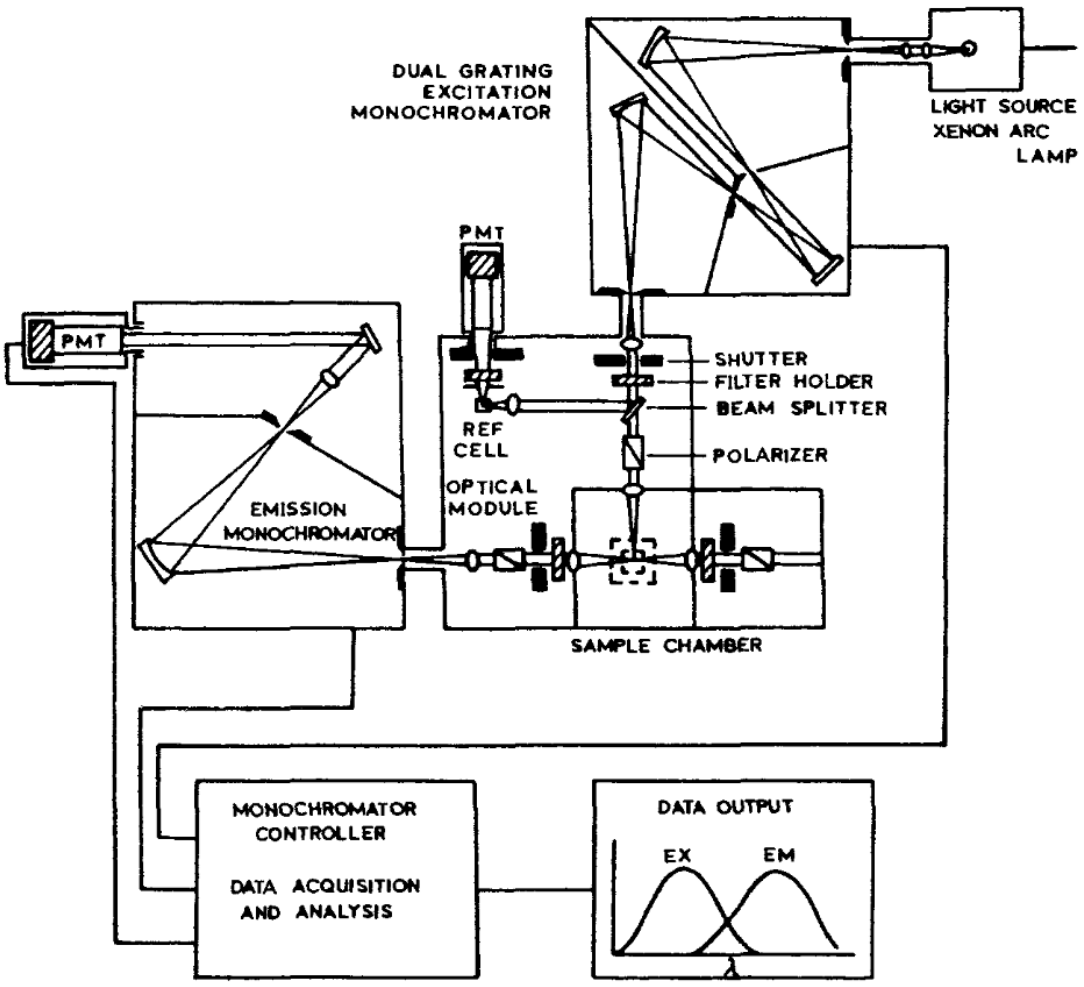


Figura 2.1: **Representacioń esquemática de un espectrofluorímetro** (**A**) Diagram of the Horiba PTI QuantaMaster hardware. Red arrows represent motors and limit switch connectors, black is BNC, blue is USB and orange represents a fiber optic. The path that light takes inside the spectrometer is represented in thick blue arrows. (**B**) and (**C**) Representation of the old and new instrumental control module respectively. (**D**) Representation of the raw signal measured from the PMT detector. (**E**) Spectrum of the sample constructed from the raw signals measured at each wavelength.

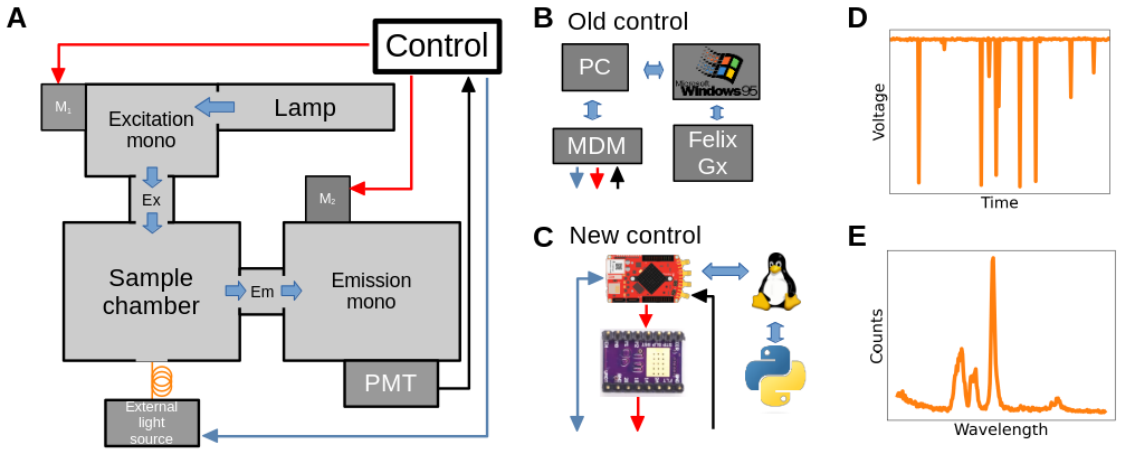


Figura 2.2: **Schematic representation of the spectrofluorometer** (**A**) Diagram of the Horiba PTI QuantaMaster hardware. Red arrows represent motors and limit switch connectors, black is BNC, blue is USB and orange represents a fiber optic. The path that light takes inside the spectrometer is represented in thick blue arrows. (**B**) and (**C**) Representation of the old and new instrumental control module respectively. (**D**) Representation of the raw signal measured from the PMT detector. (**E**) Spectrum of the sample constructed from the raw signals measured at each wavelength.

2.3. Expansión de Horiba PTI QuantaMaster 400 - Medición de tiempos de vida

introduccion a medicion de tiempos de vida

2.3.1. Hardware

Excitación con laser pulsado. Control de potencia y duty cycle. funcionamiento del trigger. el resto ya lo puede hacer la RP

2.3.2. Software

explicar que es lo mismo que antes salvo que se usa el trigger.
Explicar funcionamiento de las pantallas y offsets

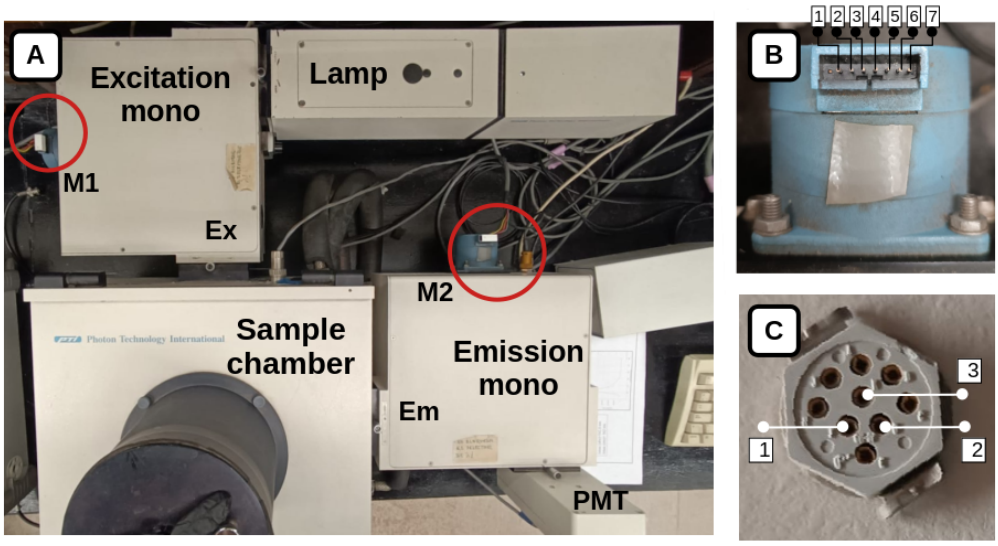


Figura 2.3: **Horiba PTI QuantaMaster 400 picture**. maybe pasar a apéndice (**A**) Picture of the whole spectrometer. Circled in red the monochromators'motors and limit switches. (**B**) Stepper motors pin diagram. The only used pins for the refurbished version are 1 and 7, and 3 and 5, which correspond to each motor winding respectively. (**C**) Limit switches pin diagram.

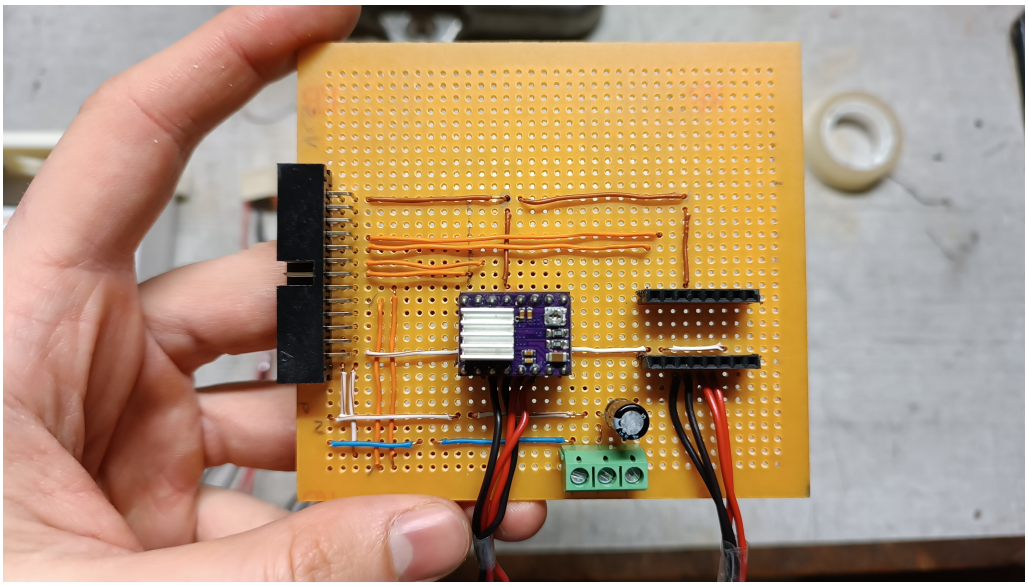


Figura 2.4: **Horiba PTI QuantaMaster 400 picture**. maybe pasar a apéndice (**A**) Picture of the whole spectrometer. Circled in red the monochromators'motors and limit switches. (**B**) Stepper motors pin diagram. The only used pins for the refurbished version are 1 and 7, and 3 and 5, which correspond to each motor winding respectively. (**C**) Limit switches pin diagram.

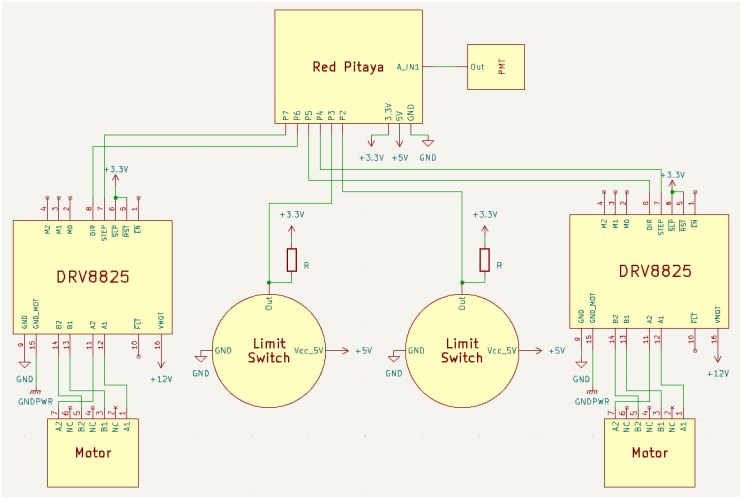
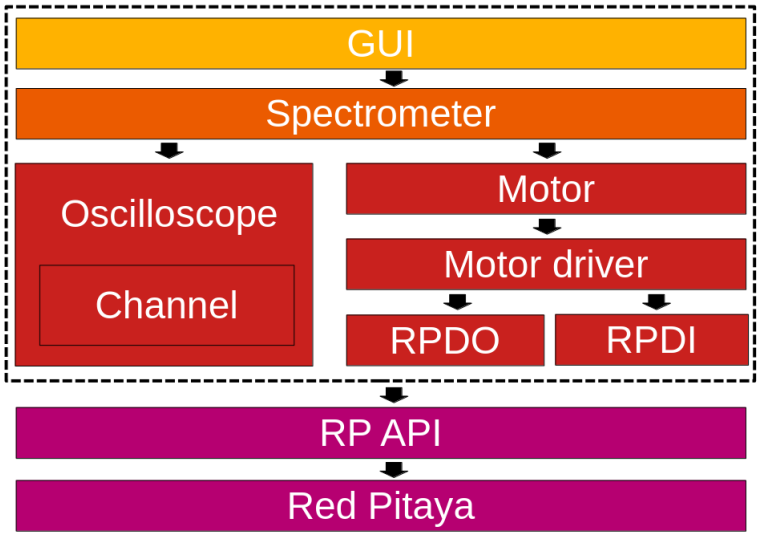


Figura 2.5: **Connection diagram**.

Figura 2.6: **Structure of the software**. Each element of the software is ordered from high level (**top**) to low level (**bottom**). Inside the dashed line black box In yellow, the two ways the end user can interact with the software. In orange, the refurbished instrument API classes. In red, the RP's hardware API.



Capítulo 3

Validación de funcionamiento

3.1. Simulación de pulsos y adquisición

3.2. Caracterización del PMT

3.3. Conteo de fotones

Capítulo 4

Caracterización de UCNPs

Capítulo 5

Conclusiones

Apéndice A

Apéndice