AROMETRIX

Beginner's Guide to Fluorescence Spectroscopy

Abstract

In 2017, Arometrix investigated and validated the use of fluorescence spectroscopy as a powerful tool for real-time molecular monitoring during short-path distillation. Our research paper, "In Situ Fluorescence Spectroscopy for In-Line Distillation Process Monitoring", was peer-reviewed and published by Cannabis Science and Technology a year later. Additionally, we had the privilege of speaking and delivering our research presentation at the Cannabis Science Conference in Maryland.

Since then, Arometrix has been a pioneer in the cannabis production industry, helping customers to optimize cannabis process control, new staff training, and batch quality. Now, **this novel technology is not only available for short-path distillation,** but also for wiped film evaporation, ethanol extraction, chromatography, conversion reaction, and soon even more.

Many of Arometrix's customers are laboratory technicians, managers, and consultants who are very knowledgeable about their processes; **however, the area of "fluorescence spectroscopy" is still a bit of a mystery to most.** It was for the purpose of advancing the knowledge in this specific field of spectroscopy that this guide was made.

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Chapter 1: An Introduction to Fluorescence Spectroscopy

What is fluorescence spectroscopy?

First, let's define each term separately. We will provide basic meanings, technical meanings, and real-life examples.

Spectroscopy

- ***Basic meaning***: The broad study of how light interacts with other light, surfaces, molecules, and other media
- ***Technical meaning:*** The branch of science concerned with the investigation and measurement of *spectra* produced when materials interact with, or emit, light
- ***Basic examples:*** Infrared (IR) cameras; Color measurements (like at Home Depot)
- ***Product examples***: Arometrix Fraction Finder (fluorescence spectroscopic measurement device); Shimadzu IRTracer-100 *(FTIR - Infrared Spectrophotometer)*; UV-1280 (Multipurpose UV-Visible Spectrophotometer); Horiba Duetta (Fluorescence and Absorbance Spectrometer)
- ***Types***: Spectrophotometry, EEMS, IR, Absorption, Raman, and Fluorescence

Fluorescence

- ***Basic meaning***: Fluorescence refers to the light produced after a wavelength is exposed to it
- ***Technical meaning***: Light radiation emitted by certain substances, usually visible light, as a result of being exposed to an external light source. This emitted light is generally of a different wavelength than the external light source.
- ***Basic examples:*** Fluorescent light bulbs, fluorescent paint, fluorescent shirts, white light LEDs

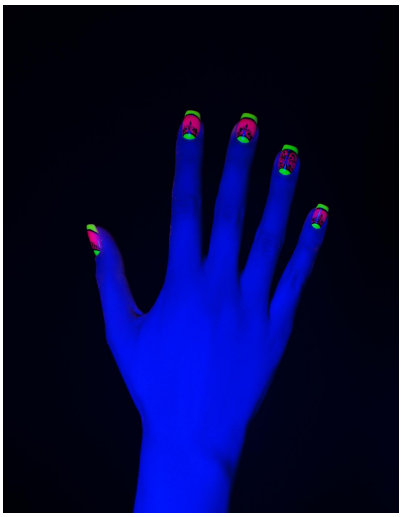


Image from DenisZbukarev

What does the "Spectra" in Spectroscopy mean?

Spectra (plural form of spectrum) is a group of different types/wavelengths of both visible and invisible light.

Basic examples include X-rays, Deep UV light, IR light, and Radio waves.

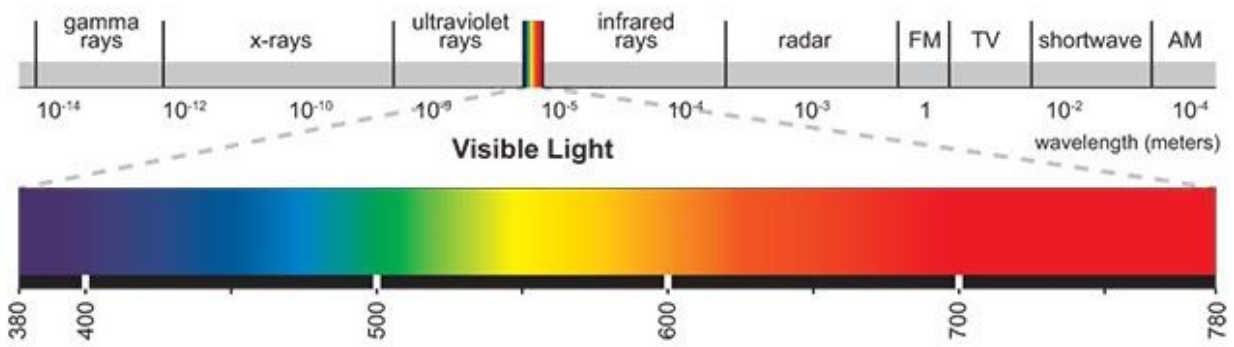
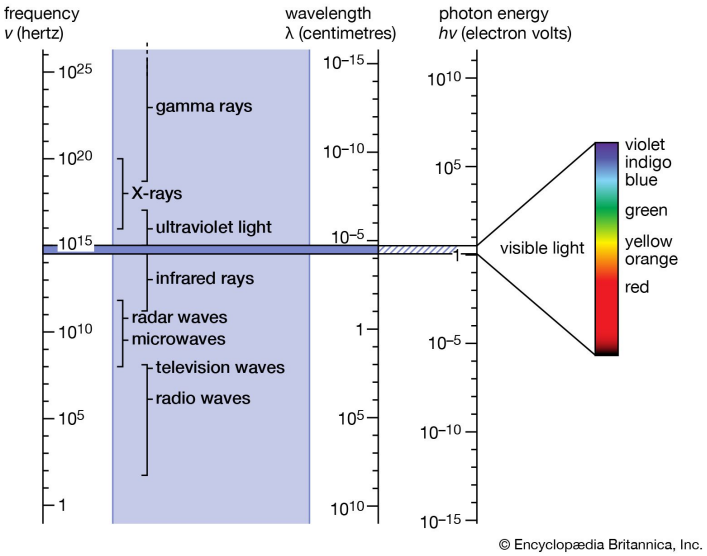


Image from Eyehortilux

What are waves, wavelengths, nanometers and peak structures?

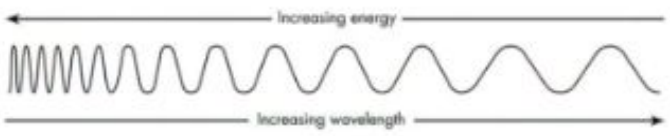
Now that we have defined the general foundation to this science, we are going to overview some key terms that, if you follow us on **Instagram**, you might have heard us mention.

A wave is a disturbance in the way in which particles move. To put this a bit more simply, light moves in waves, just as sound does; however, light's waves are different. Light's waves are classified as *transverse* waves.



Other transverse waves are ocean waves, guitar strings, and the ripple effect when you throw a rock in a lake. All of these examples have the same type of wave as light waves. Comparatively, longitudinal waves are different (i.e. sound waves).

Wavelengths are a physical aspect of a light wave. Wavelengths are the physical distance between periods of a light wave (i.e. distance between peak to peak).



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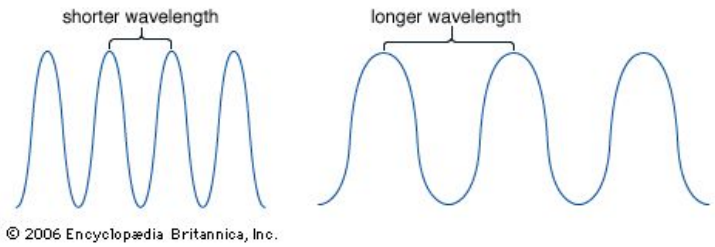


If you notice on the Fraction Finder display's Spectrum View (see below), the X-axis is wavelength.

Nanometers are a unit of length measurement with a magnitude of 10^{-9}.(eg. 1 meter is 0.000000001 nanometers). Wavelengths of light are generally represented in nanometers.

However, not *all* light is measured in nanometers.

In fact, a lot of people refer to light with eV (energy measure/photon), Hz, and even cm^(-1); the last two refer to light frequency instead of wavelength.

Peak structure refers to the shape of the spectral signal. Not *all* chemicals/molecules will have a unique Peak Structure, but they can have different, or even slightly different, peak structures which leads to them being resolvable.

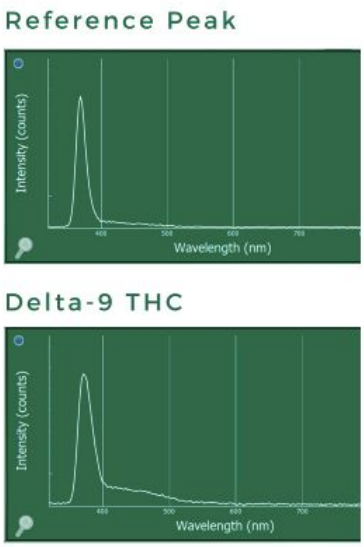


Image from Arometrix, "Chemical Cheat Sheet"

The Fraction Finder measures the amount of light at different wavelengths. The collection of these intensity values, with respect to the wavelengths, is what makes peak structures.

Putting these concepts into context: An example of these concepts can be seen in our Fraction Finder Chemical Cheat Sheet. Note how we refer to each molecule's wavelength and waveform (waveform is a colloquial term that we use to describe peak structure shape and intensity).

We've included two screenshots from the Cheat Sheet above.

- The upper image is of our Reference/Excitation peak, which is simply the internal reference peak that the system uses, showing a sharp peak structure at a wavelength region of 360-370 nanometers
- The lower image still includes the Reference/Excitation peak, but it also shows the Delta-9 THC Indicator, which displays a short broad peak structure at a wavelength region of about 450-470 nanometers.

Chapter 2: Spectroscopy Methods

Standard Light Detectors and Measurement Systems

There are several different individual detectors and whole measurement systems used for the purpose of measuring light. Below we will define the ones that are relevant to this field.

Fluorometer

- *Basic meaning*: A fluorometer is something that measures fluorescence data, specifically, the intensity of fluorescence
- *Technical measurement method*: Measures light at a right angle (90 degree) from the excitation light

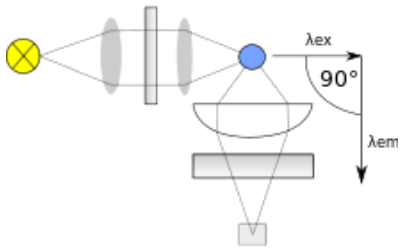


Image from Matthias M.

UV detector

- *Basic meaning*: A UV (ultra-violet) detector, commonly referred to as UV-Vis, is a detector that measures the amount of UV or visible light absorbed by components of a mixture being eluted (elution occurs during substance removal)
- *Technical measurement method*: Measures average intensity over various wavelengths; you can't distinguish between wavelengths with a UV detector.
- *What is UV?* Ultraviolet light (UV) is a domain of light, typically considered to be light with a wavelength between 10 nm to 400 nm. Basic Example: the light that you protect yourself from with sunscreen.

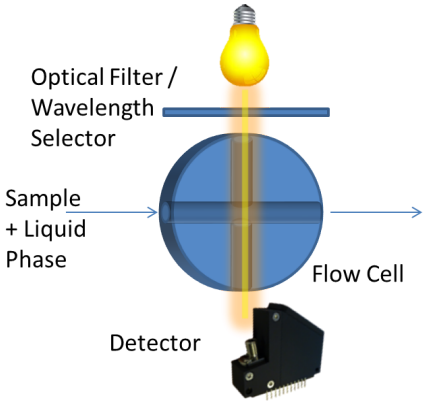


Image from Ibsen Photonics

Spectrometer

- *Basic meaning*: A spectrometer is a detector that measures spectral data. It distinguishes the wavelengths that were absorbed. Most of the time a spectrometer is a component of a spectrophotometer.
- *Technical measurement method*: Measures light absorption as a function of wavelength simultaneously.

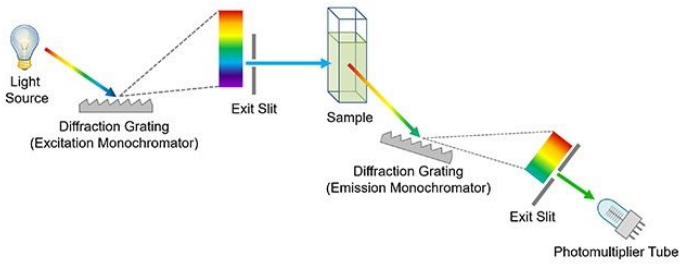


Image from Edinst

Spectrophotometers

- *Basic meaning*: A spectrophotometer is also a measurement system that measures spectral data. It focuses on the relative intensities of the wavelengths that were absorbed **or** the wavelengths that were reflected. It is a complete system that measures the absorption of light. It measures intensity as a function of wavelength one after the other.
- *Technical measurement method*: Similar to a spectrometer, but measures absorption as a function of wavelength serially.

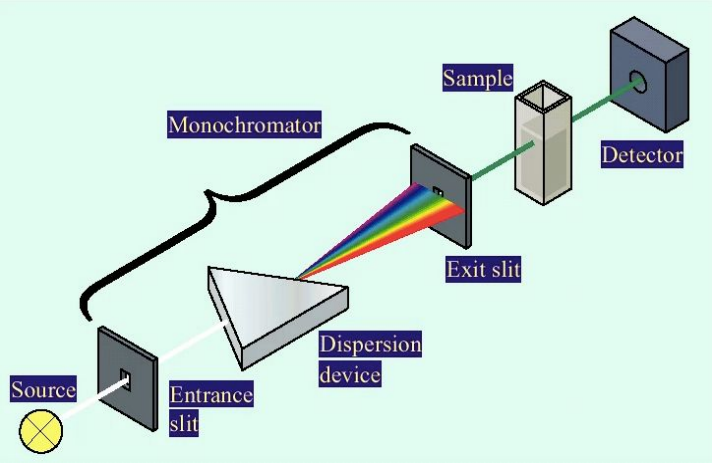


Image from Shimadzu

Furthermore, the detector's geometry is what classifies it as a specific measurement system.

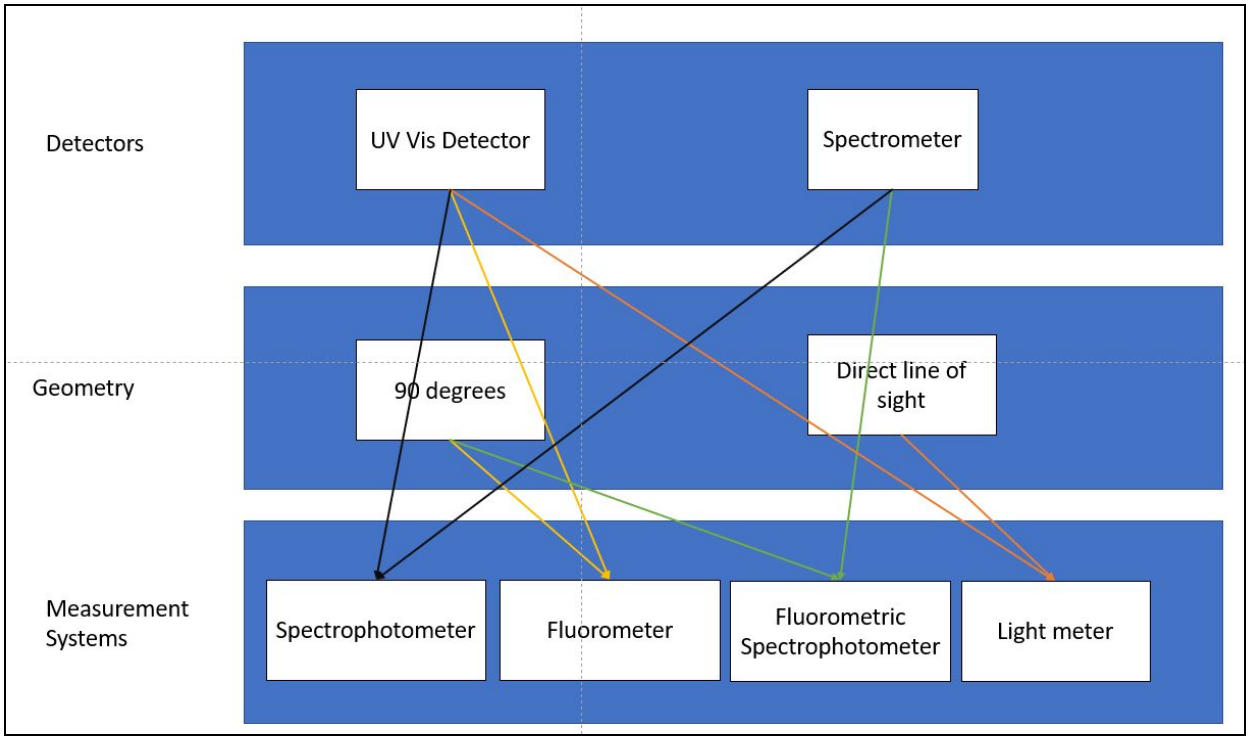


Image from Arometrix, Chris M.

It is important to list the advantages and disadvantages of the various techniques and technologies that are currently known in the cannabis analytical world. Below, we briefly evaluate each, ***in comparison to in-situ fluorescence spectroscopy (FRACTION FINDER).***

HPLC/GC - *Advantages & Disadvantages*

These typically operate with a UV detector. See the next section to learn more about the exact differences between the FRACTION FINDER and an HPLC (High Pressure Liquid Chromatography).

Advantages	**Disadvantages**
Higher level of accuracy and sensitivity - This is a quantitative measure of specific molecules	Can't be put in-line because it needs to run a physical sample through a media
Higher capabilities - More will absorb than that will fluorescence, so it can detect/distinguish more molecules	Need to actively take samples and put it through a system

Fluorometer - *Advantages & Disadvantages*

Advantages	**Disadvantages**
Reduced cost	Can't see multiple constituents, can only see one, for example you would ONLY be able to detect THC
Simple in the sense that it will give just one number	

Spectrophotometer - *Advantages & Disadvantages*

Advantages	**Disadvantages**
Could potentially measure the absorption profiles of constituents that do not fluoresce	Low-sensitivity issues - Fraction Finder is much more selective and unique to specific molecules

The Human Eye - *Advantages & Disadvantages*

This is not technically a spectroscopic technique per se; however, we are listing it here as it is the most used method.

Advantages	**Disadvantages**
Watching color changes with your eye is free	Subjective
	Indirect
	Not repeatable → many variables can change color
	Can't keep data records
	Requires a great deal of experience and trial & error

What is the difference between Fraction Finder and HPLC?

We get this question a lot. While both technologies use spectroscopic analysis for high sensitivity and detection of specific molecules, there are three key differences.

Key Differences

Different types of spectroscopy detection methods	Fraction Finder utilizes induced *fluorescence* spectroscopy. HPLC utilizes *absorption* spectroscopy. ● Absorption is light that undergoes an energy transformation (typically from photon energy to thermal energy). An example of this is putting a blue filter sheet in front of a white light; all light that is not blue is absorbed by the filter (this is very common in performance theatre). ● Fluorescence is light that was transmitted from a media/material due to chemical, electronic, or energy change of that media due to an interaction with a different wavelength of light. An example of this is the Fraction Finder's sensor measurements.
Different UV regimes	Fraction Finder operates in the near UV. Most HPLCs operate in the deep UV. ● Fluorescence (in the Fraction Finder's case) operates in the near UV, which consists of shorter wavelengths than the wavelengths that humans can see ● HPLCs operate in the deep UV, which consists of longer wavelengths than the wavelengths that humans are able to see ● Fluorescence *can* occur in the UV (ultra-violet) but is completely separate from UV. Think about these terms more like car colors and makers. You can buy a black Ford Fiesta, but you can also buy a black BMW, or you could buy a white Ford Fiesta. The car color and maker are both aspects of a car but they don't really directly relate with each other.
Different use cases	Fraction Finder is used for in-line quality monitoring *during* the process. HPLCs are used for sample analysis *after* the process. ● HPLC is the traditional method of quantifying the mass distribution of specific molecules in a given sample. ● In the cannabis industry, this is what analytical laboratories typically use to accept and reject samples.

Chapter 3: Overview of Relevant Key Concepts

Electromagnetic Radiation

Basic meaning: Spectroscopy uses spectra in the investigation of electromagnetic (EM) radiation. It is the interaction of, and emission of, light.

Technical meaning: This relates to the interrelation of electric currents or fields and magnetic fields. Specifically, this is defined as a fundamental physical force that is responsible for interactions between charged particles, which occur because of their charge and for the emission and absorption of photons, that is about a hundredth the strength of the strong force, and that extends over infinite distances but is dominant over atomic and molecular distances. Radiation refers to the emission of energy as electromagnetic waves or as moving subatomic particles, especially high-energy particles which cause ionization. In other words, it refers to the way that electrically charged particles radiate, or scatter, upon interaction.

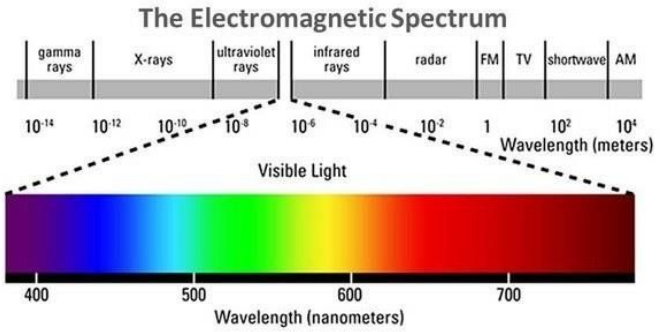


Image from E. Campostrini

Spectrum

The full range of wavelengths of electromagnetic radiation

Waves & Electromagnetic Radiation

The way in which charged particles interact and oscillate (or vary in magnitude or position) in magnetic fields. More specifically, this is a variation of an electromagnetic field in the propagation of light or other radiation through a medium or vacuum. This periodic disturbance of the particles of a substance may be propagated without net movement of the particles.

Light Scattering

Light that has been diffused through a media (i.e. something in) and (at least partially) re-emitted (i.e. something out).

A real-life example of this is a prism.

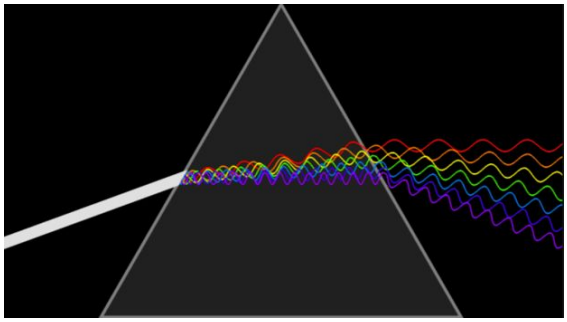
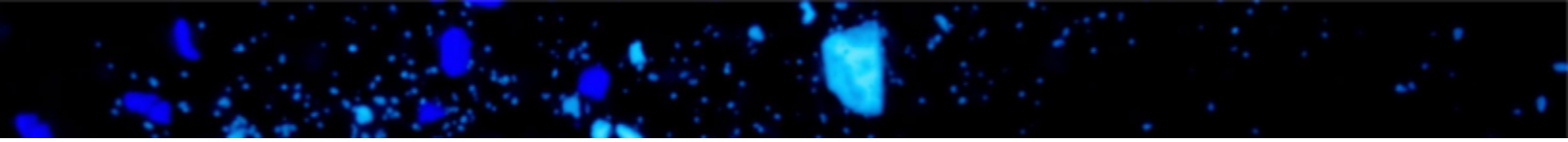


Image from Jochem Vreeman



Fluorescence is a light scattering event because light is being re-emitted. Furthermore, it is an inelastic scattering phenomena where light is emitted from a sample due to electron excitation and relaxation by a specific energy of light.

With **inelastic scattering**, during a given particle collision, energy is absorbed by one or more particles. Examples of this include Fluorescence and Raman scattering. Comparatively, with **elastic scattering**, during a given particle collision, energy is not absorbed by either particle. An example of this is any type of diffraction, such as X-Ray. Even the formation of rainbows is inherently an elastic process because the light doesn't change its energy, just its direction.

Excitation

The application of energy to a particle, object, or physical system. In other words, this is considered the addition of energy, so exciting something means applying/adding energy to it. The state that a particle is in during excitation (when an atom or molecule has absorbed energy) is referred to as the **excited state.**

Photon

A particle representing a quantum of light or other electromagnetic radiation

Transmission vs Reflection

Transmission refers to the light that has passed straight through a media. An example of this is visible light passing straight through a clean window. **Reflection** refers to the light that bounces off an interface. An example of this is visible light bouncing off a mirror.

Summary

Arometrix hopes that this guide has been educational to those who are beginners in the field of fluorescence spectroscopy. Moving forward, Arometrix plans to continuously update this document as we receive more questions and comments on the subject matter. With that said, if you would like to discuss this paper, learn more about our technology, or get a product quote, please do not hesitate to get in touch with our team. We love to hear from you!

Get In Touch

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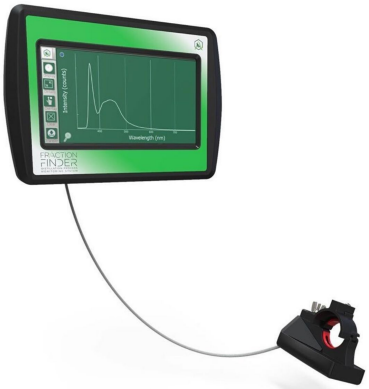
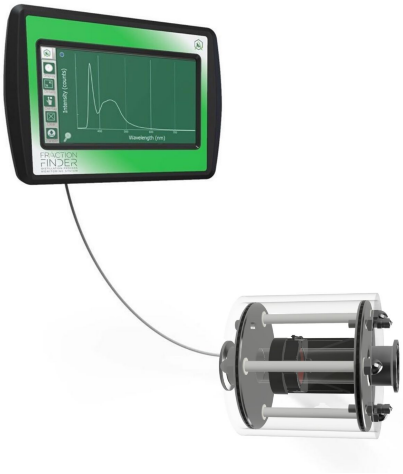
- Instagram: @arometrix

FRACTION FINDER



EXTRACTION FINDER



FRACTION FINDER **ULTRA**