HOW TO:
RAMAN AND FLUORESCENCE SPECTROSCOPY

A BRIEF OUTLOOK on DEVELOPMENTS in RAMAN SPECTROSCOPY INSTRUMENTATION

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Graz, 22.09.2022

PA GRAZ 2022

https://carlahub.eu/events/pa-graz/

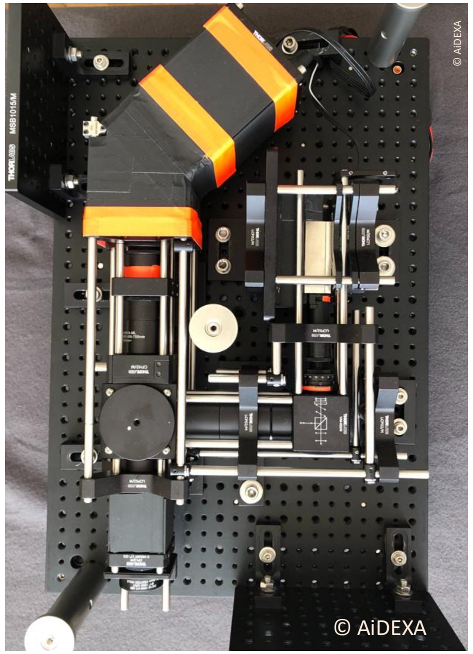
INTRODUCTION: i & AiDEXA GmbH

AiDEXA is a boutique builder of
optical spectroscopy instrumentation

BRIEF CASES DESCRIPTION – EXPERIMENTAL WiP

eRPHiX

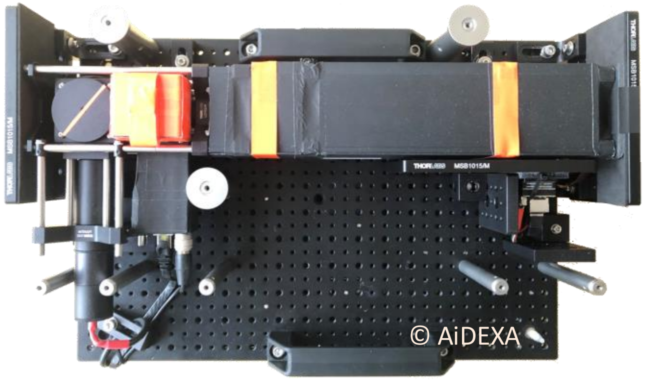
experimental
Raman
Plasmonic
Hyperspectral
Imaging



eRPHiX is used to visualize a hyperspectral imaging cube, the continuous scan of a colloidal droplet or nano-substrate

eFLUORiX

experimental
Fluoride
Spectroscopic
Instrumentation



eFLUORiX is used to quantify low concentrations of fluoride in water vapor exhaust from a fuel cell by means of spectroscopically resolved absorbance.

BASICS OF SPECTROSCOPY

- INTRODUCTION AiDEXA
- BRIEF CASES DESCRIPTION
- **BASICS OF SPECTROSCOPY**
- BUILDING BLOCKS OF OPTICAL SPECTROSCOPY INSTRUMENTATION
- CONCLUSION, RECOMMENDED LITERATURE

Spectroscopy studies the interaction of electromagnetic radiation with matter, where this interaction is measured to obtain **quality and quantity of the chemical species.**

BASICS OF SPECTROSCOPY – SOME HISTORY x.OPTICS

Source: as pictured at the KFU Graz

The first modern application of optics occurred in Florence around 1280 A.D. with the use of eyeglasses as an aid to vision.

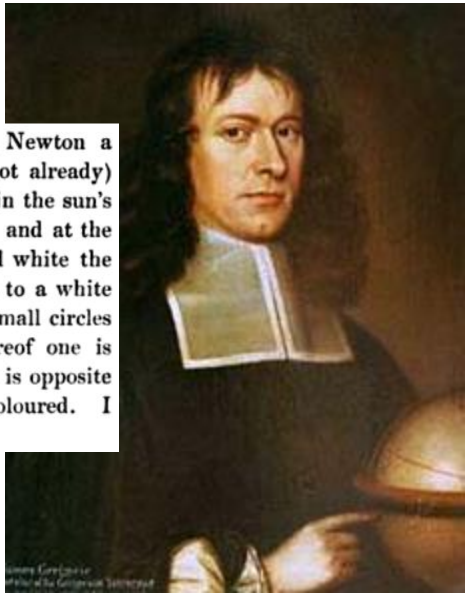
Antoni van Leeuwenhoek (1632-1723)

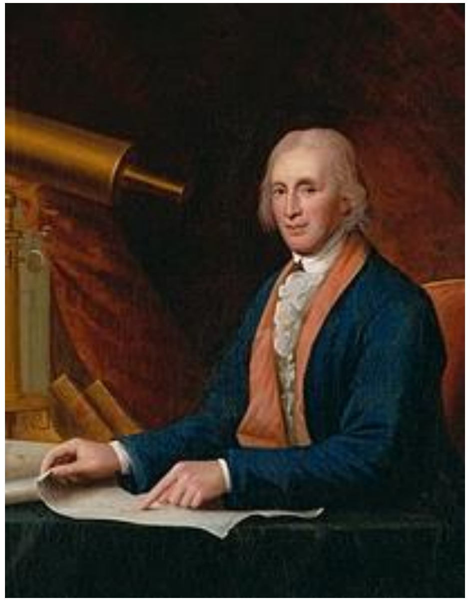
The lens is a sphere approximately 2 mm in diameter giving a magnification of about 180 x.

BASICS OF SPECTROSCOPY – SOME HISTORY.DIFFRACTION

If ye think fit, ye may signify to Mr. Newton a small experiment, which (if he know it not already) may be worthy of his consideration. Let in the sun's light by a small hole to a darkened house, and at the hole place a feather, (the more delicate and white the better for this purpose,) and it shall direct to a white wall or paper opposite to it a number of small circles and ovals, (if I mistake them not,) whereof one is somewhat white, (to wit, the middle, which is opposite to the sun,) and all the rest severally coloured. I would gladly hear his thoughts of it.

Source: Letter from James Gregory to John Collins, dated 13 May 1673, https://en.wikipedia.org/wiki/James_Gregory_(mathematician)#cite_note-20





Sir Isaac Newton
(1643 –1727)

James Gregory
(1638 –1675)

David Rittenhouse
(1732 –1796)

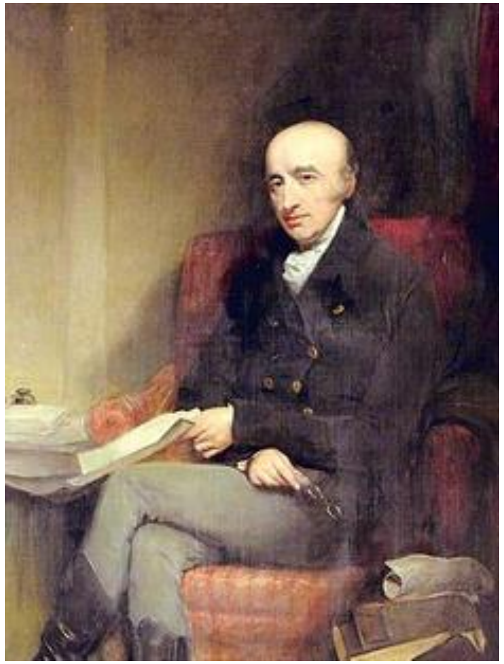
REFRACTION PRISM

THE FIRST DIFFRACTION GRATING
(in a natural form)

1785, Rittenhouse made the **first diffraction grating** using **50 horse hairs** between two finely threaded screws, with an approximate spacing of about 100 lines per inch (ca. **4 lines / mm**).

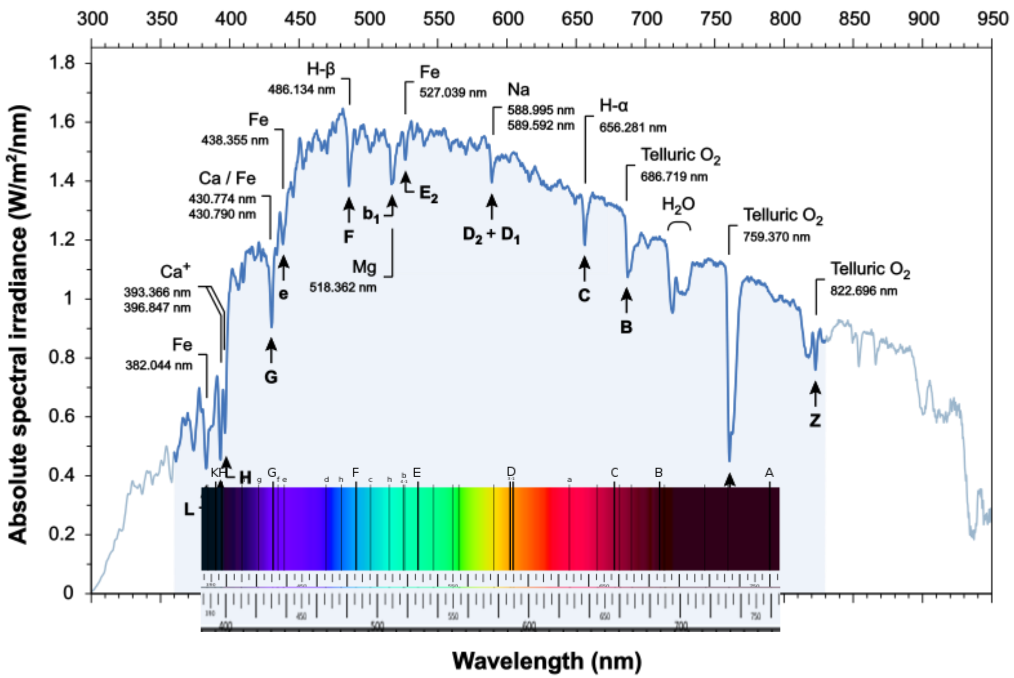
BASICS OF SPECTROSCOPY – SOME HISTORY.SPECTRUM

From Newton's **round opening** and **prism**
to **SLIT** and **PRISM** – **NON-linear - REFRACTION**
to **SLIT** and **GRATING – Linear - DIFFRACTION**

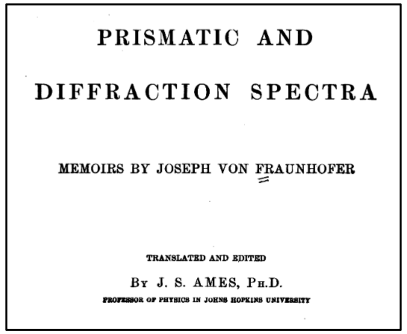


William Hyde Wollaston
(1766-1828)

Joseph von Fraunhofer
(1787–1826)



[Prismatic and diffraction spectra](): memoirs. By Joseph von Fraunhofer, William Hyde Wollaston. American Book Co., 1899.



PRISMATIC AND

DIFFRACTION SPECTRA

MEMOIRS BY JOSEPH VON FRAUNHOFER

TRANSLATED AND EDITED

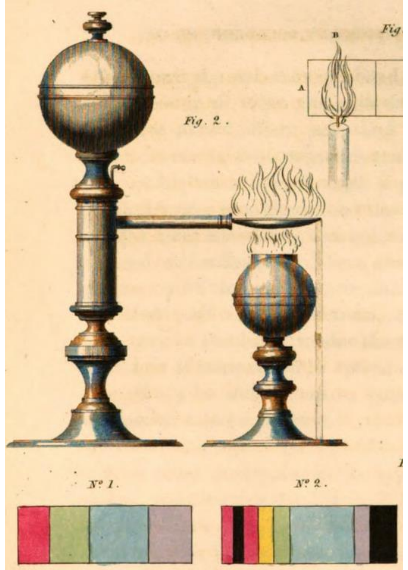
BY J. S. AMES, PH.D.
PROFESSOR OF PHYSICS IN JOHNS HOPKINS UNIVERSITY

BASICS OF SPECTROSCOPY – SOME HISTORY.FLUORESCENCE

OPTICAL SPECTRA PHOTOGRAPHY

Sir John F. W. Herschel
(1792 – 1871)

Reproduced courtesy of the Library and Information Centre, Royal Society of Chemistry

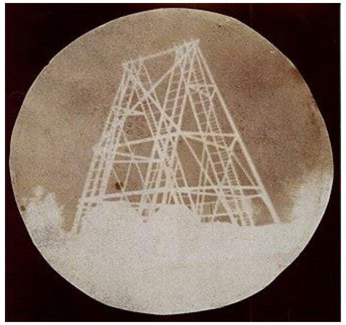


In **1823**, Herschel published his findings on the **optical spectra of metal salts**. "On the Absorption of Light by Coloured Media …and on the Colours Exhibited by Certain Flames…."

Source: Transactions of the Royal Society of Edinburgh. ... 9 (1823). https://babel.hathitrust.org/cgi/pt?id=hvd.hwhq76&view=1up&seq=515&skin=2021

In **1845**, he reported the first observation of **fluorescence from a quinine solution in sunlight,** *it differs from scattered light*

Herschel made numerous important contributions to photography. He discovered in **1819** sodium thiosulfate **to "fix" pictures and make them permanent.**
He coined the term **photography** in 1839; was the first to apply the terms negative and positive to photography.



J. Herschel's first glass-plate photography, dated 09.09.1839, showing the mount of his father's 40-foot telescope

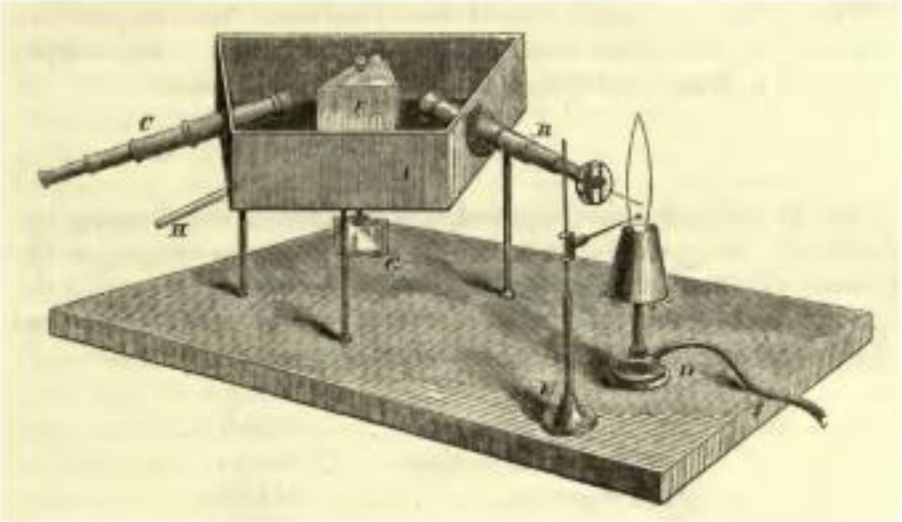
Brief History of Sir. John Fredrich William Herschel: source Principles of Fluorescence Spectroscopy by Joseph R. Lakowicz

BASICS OF SPECTROSCOPY – SOME HISTORY.SPECTROSCOPE

Gustav Kirchhoff (left) and Robert Bunsen (right)

Gustav Kirchhoff (1824-1887)

Robert Bunsen (1811-1899)



First spectroscope of Kirchhoff and Bunsen.

Source: M. João Carvalhal, Manuel B. Marques, "Adam Hilger revisited: a museum instrument as a modern teaching tool," Proc. SPIE 9793, Education and Training in Optics and Photonics: ETOP 2015, 979328 (8 October 2015); doi: 10.1117/12.2223202 Event: Education and Training in Optics and Photonics: ETOP 2015, 2015, Bordeaux, Franc

they demonstrated that **EACH CHEMICAL ELEMENT HAS A UNIQUE SPECTRUM** and established spectroscopy as a powerful method of analysis

Source: as pictured at the KFU Graz

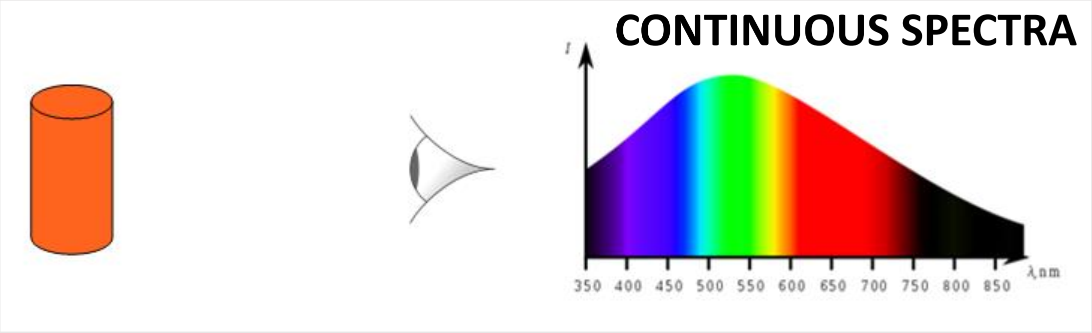
BASICS OF SPECTROSCOPY – KiRCHHOFF's LAWS of SPECTR.

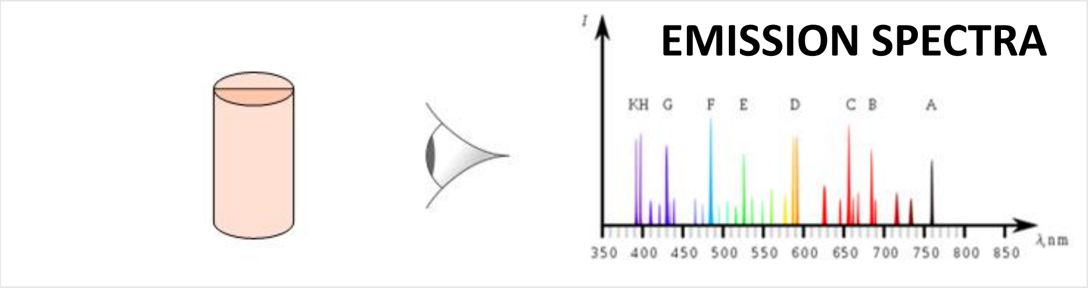
KIRCHHOFF'S LAWS OF SPECTROSCOPY describe the spectral composition of light emitted by INCANDESCENT OBJECTS :

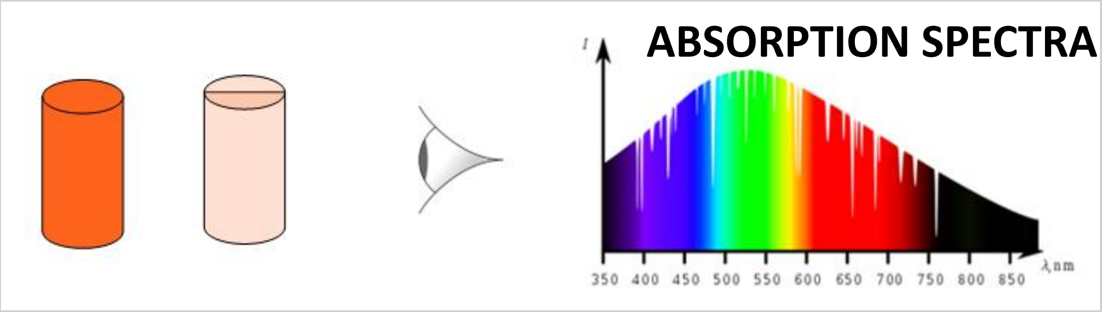
1. A **CONTINUOUS SPECTRUM** will be radiated by a solid, liquid, or dense gas excited to emit light.

2. A low-density gas excited to emit light, will do so at specific wavelengths producing an **EMISSION SPECTRUM.**

3. If light with a continuous spectrum passes through a cool, low-density gas, the result will be an **ABSORPTION SPECTRUM**.



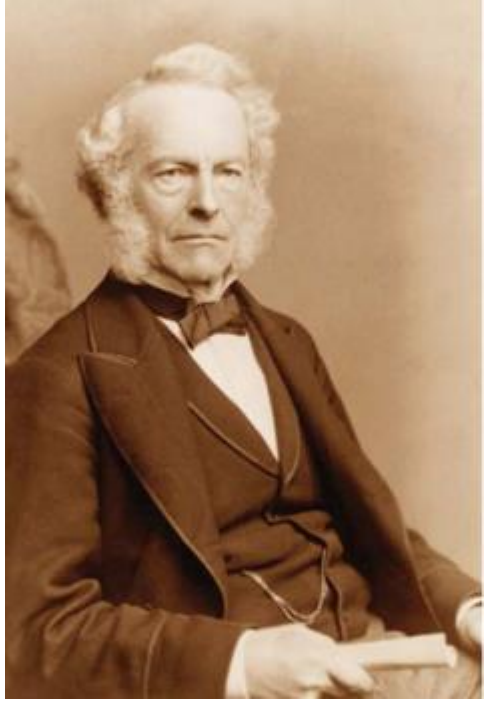




Adapted from Source: https://commons.wikimedia.org/w/index.php?curid=65526749

BASICS OF SPECTROSCOPY – SOME HISTORY.FLUORESCENCE

STOKES SHIFT

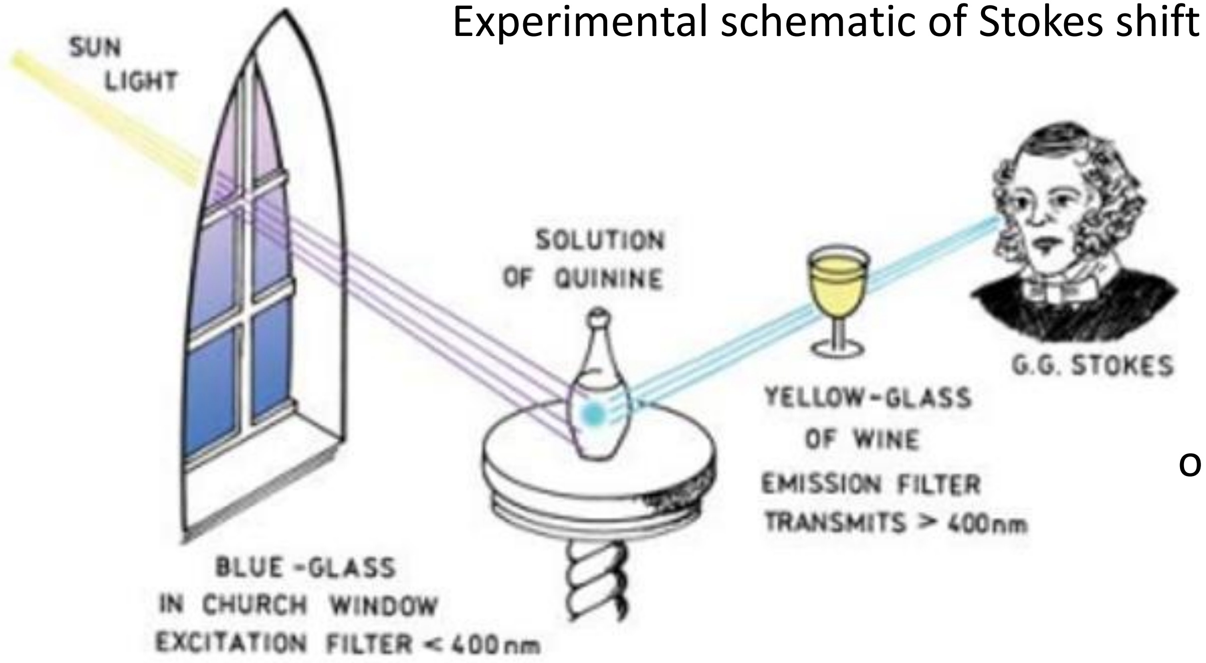


Sir George Gabriel Stokes
(1819 – 1903)

Reproduced courtesy of the Library and Information Centre, Royal Society of Chemistry

At the Cambridge U. in 1852, **Sir Stokes** found that **emitted fluorescent light** was **composed of wavelengths LONGER than those of the absorbed light and lower energy –** calling this the **STOKES SHIFT.**

Experimental schematic of Stokes shift



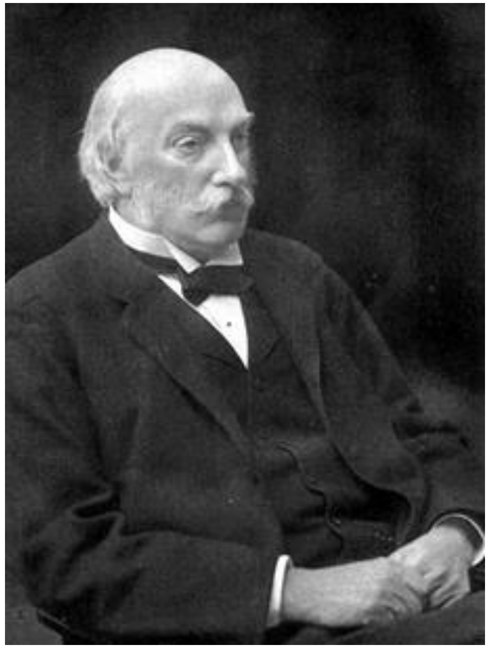
Stokes later suggested using optical properties **to identify substances**

Quinine fluorescence occurs near 450 nm and is therefore easily visible.

Source: Principles of Fluorescence Spectroscopy by Joseph R. Lakowicz

BASICS OF SPECTROSCOPY – ELASTIC SCATTERING

RAYLEIGH SCATTERING



John William Strutt,
Lord Rayleigh
(1842 – 1919)

When light passes through matter of any kind it is either transmitted, absorbed or passes onwards in a process called **SCATTERING.**

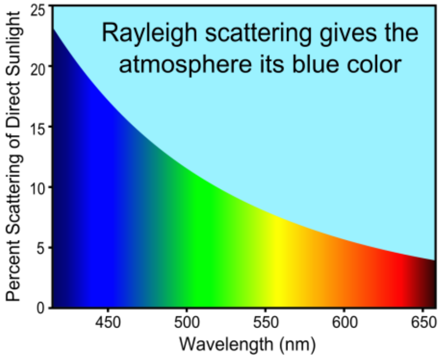
When there is **NO LOSS OF ENERGY** of the scattered light the process is called **ELASTIC** or **RAYLEIGH SCATTERING**

Rayleigh scattering applies **to particles that are small** with respect to wavelengths of light, below roughly 40 nm (for visible light) and the particles may be individual atoms / molecules.

It results from the electric polarizability of these particles. The oscillating electric field of a light wave acts on the charges within a particle, **causing them to oscillate at the same frequency.**

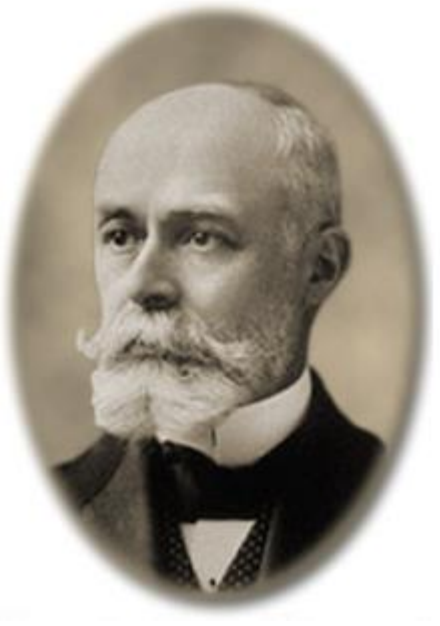
$$I = I_0 \left(\frac{1+\cos^2\theta}{2R^2} \right) \left(\frac{2\pi}{\lambda} \right)^4 \left(\frac{n^2-1}{n^2+2} \right)^2 \left(\frac{d}{2} \right)^6,$$

Intensity of scattering is inversely proportional to the fourth power of the wavelength (**~λ^{-4}**)

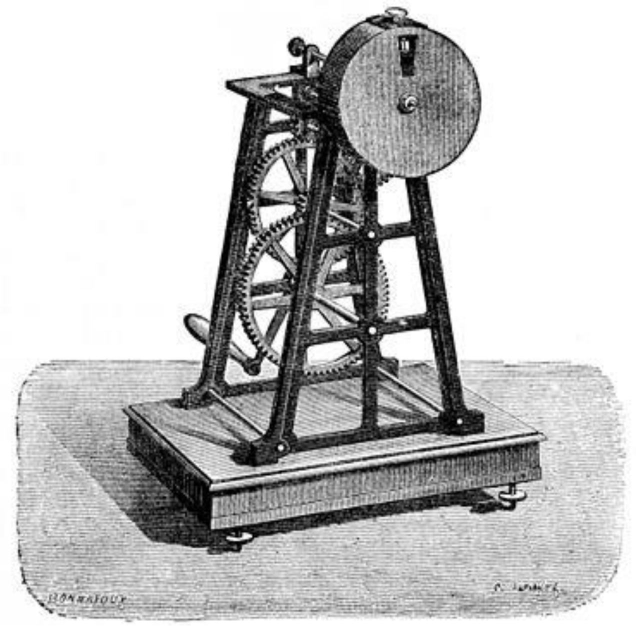


BASICS OF SPECTROSCOPY – SOME HISTORY.PHOTOGRAPHY

The appearance of photography offered the possibility of recording the observations permanently - **the first photography of a spectrum** can be traced down to 1842, when the French physicist Alexandre-Edmond Becquerel placed a daguerreotype plate in front of a solar spectrum to record its image.



Alexandre-Edmond Becquerel
(1820 – 1891)



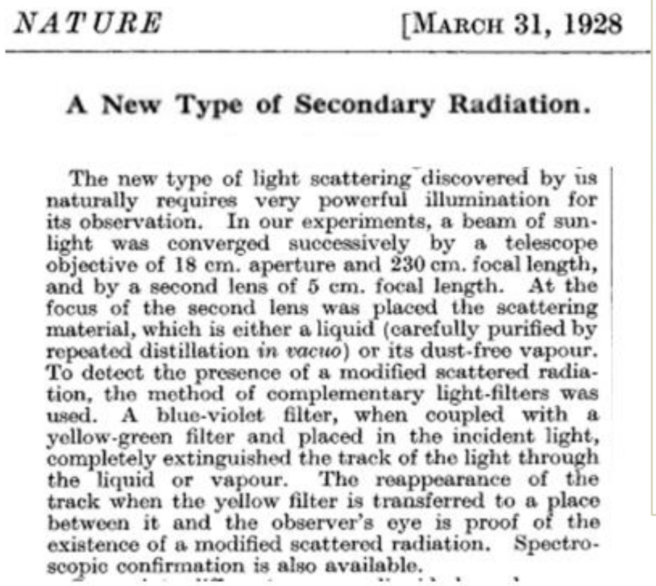
The Becquerel phosphoroscope, 1858
measurement of decay times > 1ms

Source: as pictured at the KFU Graz

La lumiére, ses causes et ses effets

BASICS OF SPECTROSCOPY – SOME HISTORY - RAMAN

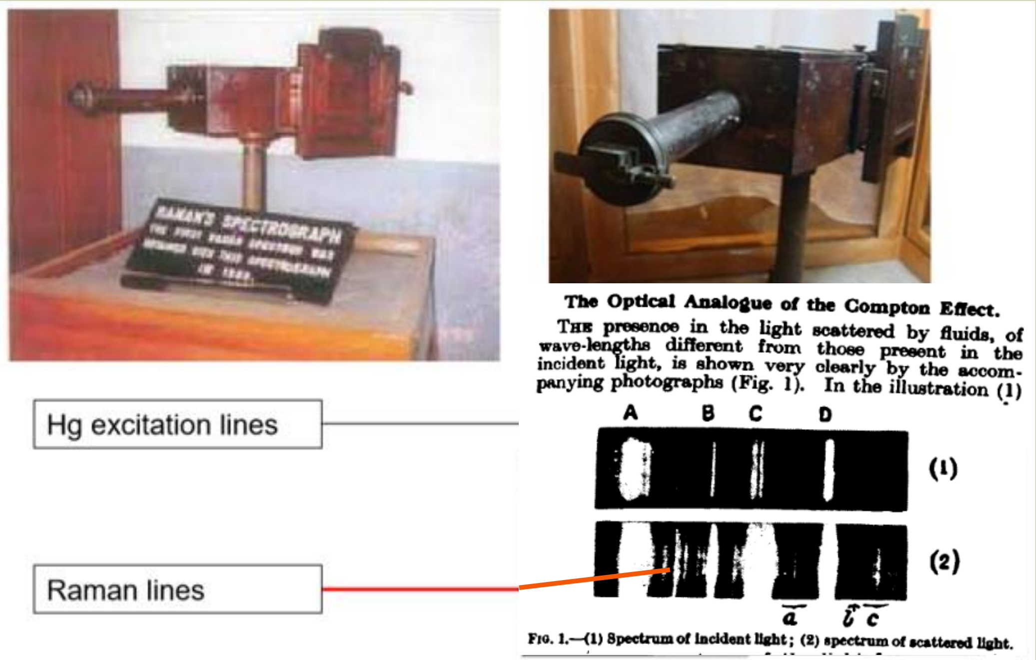
1923 Compton effect: **inelastic** X-Ray **scattering**; for ordinary light predicted by A. Smekal



NATURE [MARCH 31, 1928

A New Type of Secondary Radiation.

The new type of light scattering discovered by us naturally requires very powerful illumination for its observation. In our experiments, a beam of sunlight was converged successively by a telescope objective of 18 cm. aperture and 230 cm. focal length, and by a second lens of 5 cm. focal length. At the focus of the second lens was placed the scattering material, which is either a liquid (carefully purified by repeated distillation *in vacuo*) or its dust-free vapour. To detect the presence of a modified scattered radiation, the method of complementary light-filters was used. A blue-violet filter, when coupled with a yellow-green filter and placed in the incident light, completely extinguished the track of the light through the liquid or vapour. The reappearance of the track when the yellow filter is transferred to a place between it and the observer's eye is proof of the existence of a modified scattered radiation. Spectroscopic confirmation is also available.



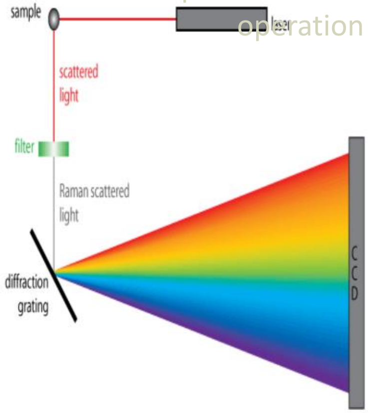
Source: EMU-CNRS International School: Applications of Raman Spectroscopy to Earth Sciences and cultural Heritage, June 2012

Sir **C. V. Raman**
Chandrasekhara Venkata Raman
(1888 – 1970)

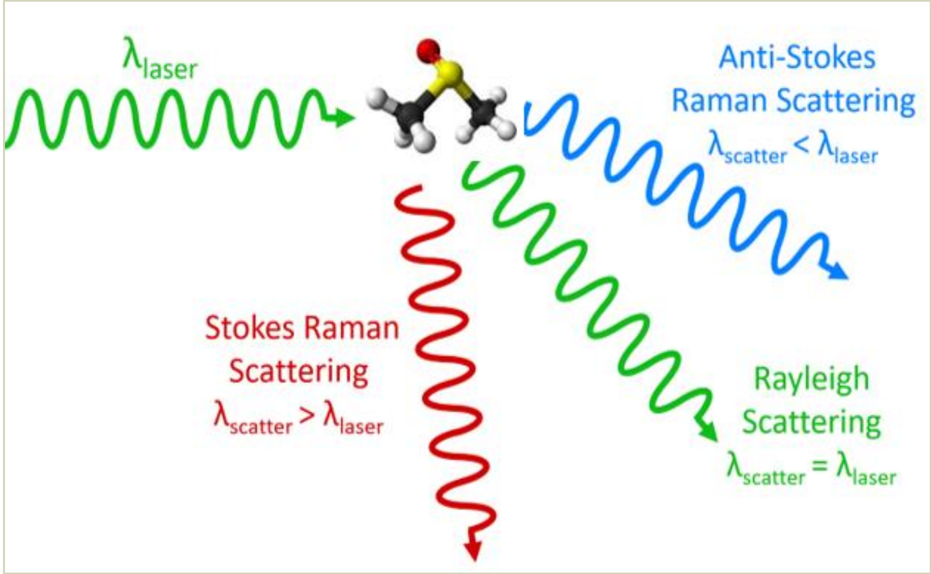
In 1928 C.V. Raman and K.S. Krishnan experimentally confirmed that the visible wavelength of a small fraction of the radiation scattered by certain molecules **differs from that of the incident beam** and furthermore that the **shifts in wavelength depend upon the chemical structure of the molecules** responsible for the scattering.

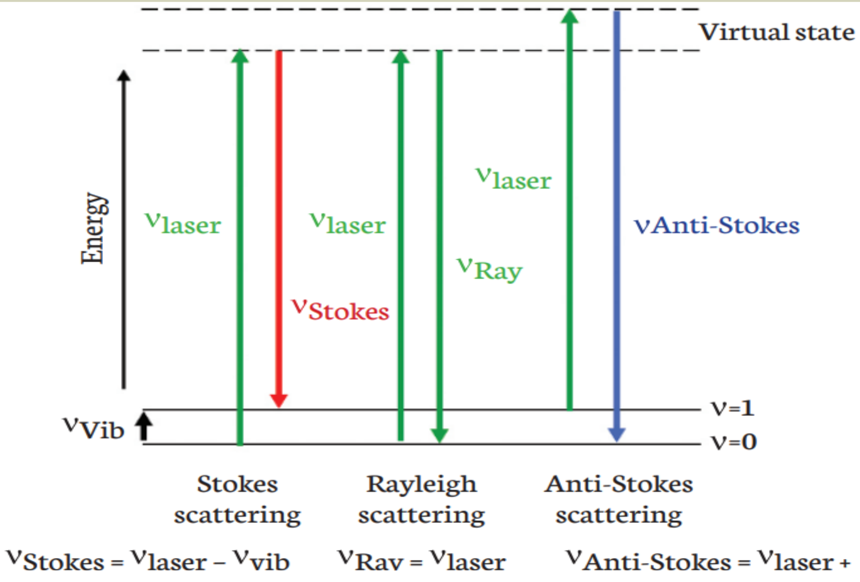
BASICS OF SPECTROSCOPY – RAMAN SCATTERING

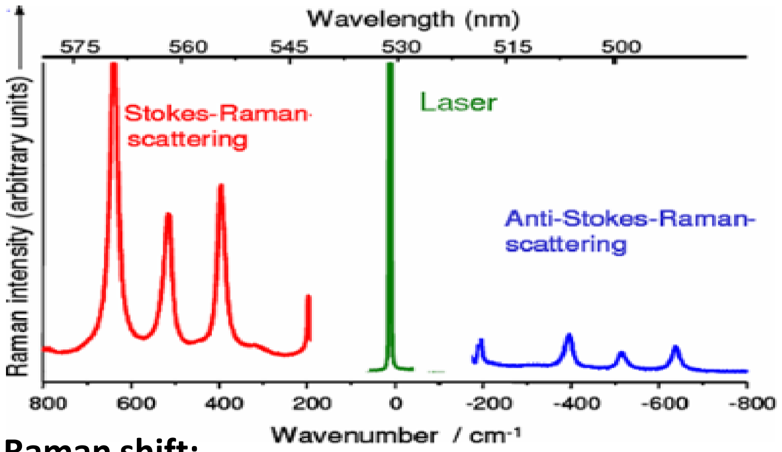
A simplified diagram of a Raman spectrometer's operation



https://www.doitpoms.ac.uk/tlplib/raman/printall.php







Stokes and anti-Stokes Raman spectra of anatase (TiO_2)
Source: N. Tarcea et al. in Raman Spectroscopy—A Powerful Tool for in situ Planetary Science, in Space Sci Rev (2008) 135: 281–292, DOI 10.1007/s11214-007-9279-y

In Raman spectroscopy, an intense, monochromatic beam of electromagnetic radiation is focused on the sample, and the intensity of the scattered radiation is measured as a function of its wavelength.

Usually, in a Raman spectrum the intensity is plotted as a function of the Raman wavenumber ω, expressed in cm^{-1}, which is related to the difference in frequency between the scattered light and the incident electromagnetic radiation

Raman shift:
relative wavenumbers with respect to the incident excitation radiation

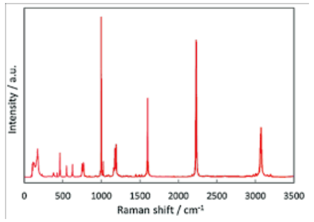
BASICS OF SPECTROSCOPY – RAMAN SCATTERING

Knowing how much energy the molecules absorb provides information about the **chemistry and physical state of the sample** such as the types of covalent bonds, the electronic state, the crystallinity and the degree of stress present in the sample.

Thus, a Raman spectrum contains the information on:
material identity and composition = char. Raman bands;
material quantities = peak intensities and MVA scores;
degree of crystallinity or phase = peak width and shape;
crystal symmetry and orientation = polarization state.

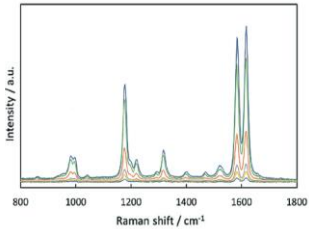
Raman scattering is a function of the polarizability of the molecular bond, or how easy it is to move electrons within the molecule by the electric field of the light.

POSITION of PEAKS



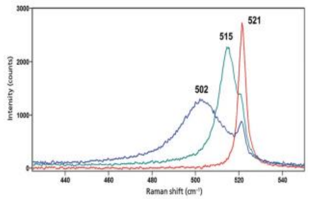
→ **MATERIAL IDENTITY & MIXTURES**

INTENSITY of PEAKS



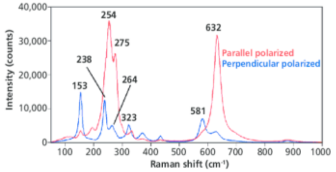
→ **MATERIAL QUANTIFICATION**

PEAK SHIFT & WIDTH



→ **STRESS & STRAIN**

PEAK POLARIZATION

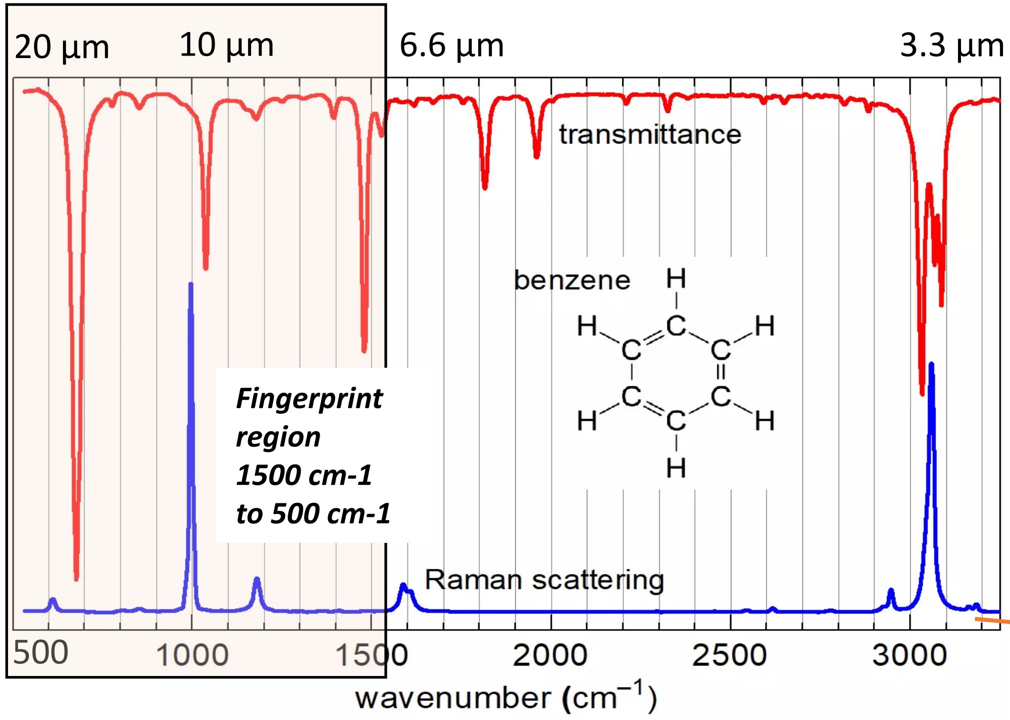


→ **CRYSTAL SYMMETRY**

BASICS OF SPECTROSCOPY – RAMAN - WHY SO SPECIAL

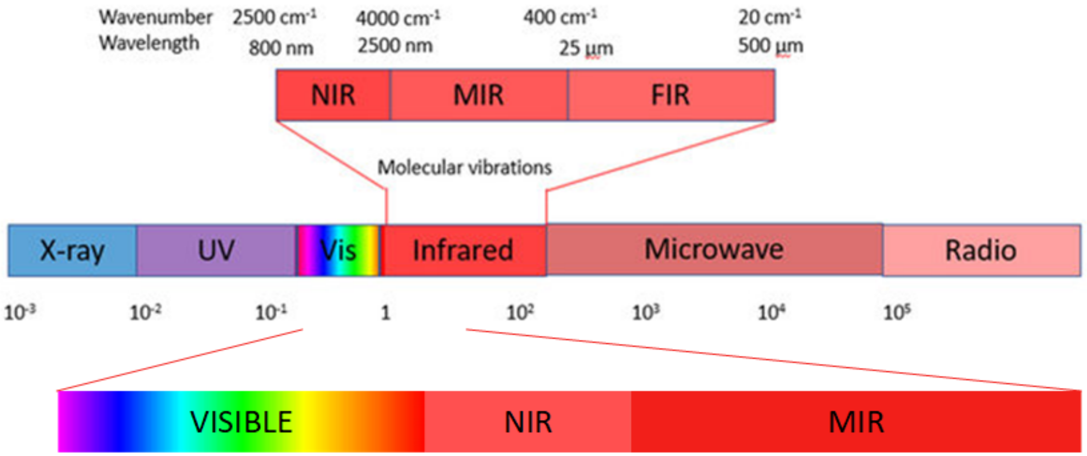
Measurement of molecular vibrations and fingerprint:

- either direct as IR absorption
- or indirect Raman scattering



Source: https://www.smacgigworld.com/blog/difference-between-ir-and-raman-spectroscopy.php

Wavenumber [cm^{-1}] = 10000 / wavelength[µm]



Raman Scattering moves information to shorter wavelengths

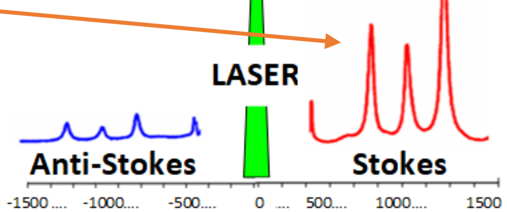
Molecular Bond Fingerprint information is in this range 1500cm-1 – 500cm-1

(+) Low cost optics
(+) HiRes detector arrays
(+) No water absoption
(-) Fluorescence

Raman Bond information similar to FTIR

FTIR sees this region

Strongly affected by H_20 absorption



Source: https://www.researchgate.net/profile/R-Hochleitner/publication/41625128_Raman_SpectroscopyA_Powerful_Tool_for_in_situ_Planetary_Science/links/00b495346e7afa1d1a000000/Raman-SpectroscopyA-Powerful-Tool-for-in-situ-Planetary-Science.pdf?origin=figuresDialog_download

BASICS OF SPECTROSCOPY – SOME HISTORY – LIGHT SOURCE

"I was the only one that analyzed ruby in enough detail to have the confidence to stick with it."

Theodore Harold Maiman
(1927 – 2007)

1928	1960	1962	1965	2000	2005
SUN Hg ARC DISCHARGE Lamp	Townes et. al. Suggest LASER Maiman makes it work	First use of Ruby Laser for Raman by Porto + Wood	Weber + Porto He-Ne for HiRes Raman Spectroscopy	Nd-Yag 532nm HiRes Raman Spectroscopy	Laser Diodes

Source: Reproduced after Colthup, N. - Introduction to Infrared and Raman Spectroscopy, 1975

BASICS OF SPECTROSCOPY – SOME HISTORY

By the end of the 1930s, Raman spectroscopy had become a mainstream method for non-destructive chemical analysis, mostly used for the analysis of bulk samples.

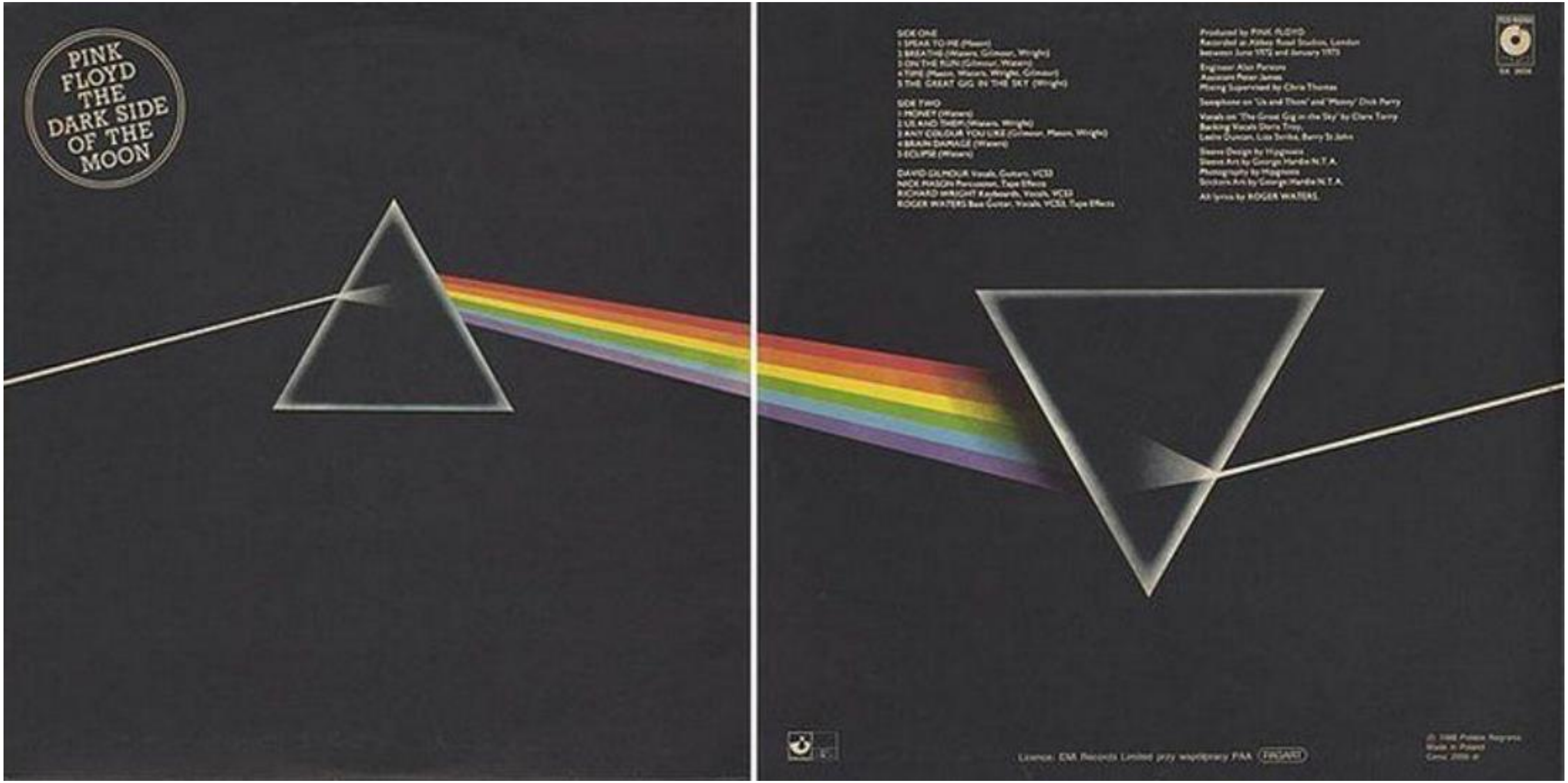
The proliferation of lasers, better optical filters and optics even microscopy (**Delhaye and Dhamelincourt in 1975**) and more sensitive detectors have allowed the growth of Raman Spectroscopy / MicroSpectroscopy.

But one weakness remained….

"the excessive feebleness of the effect"

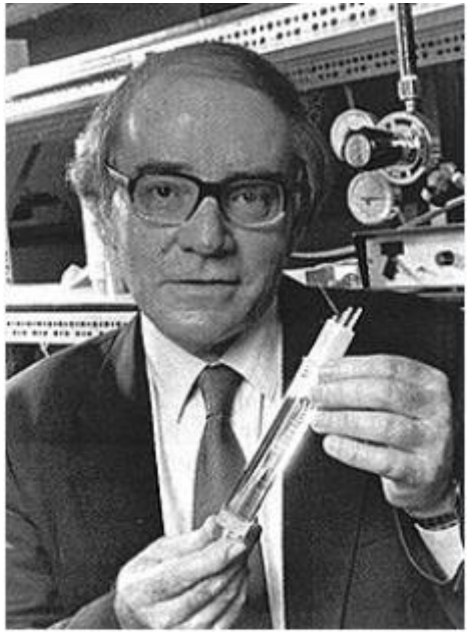
(Nature,1928) - the poor scattering signal

BASICS OF SPECTROSCOPY – SOME HISTORY



BASICS OF SPECTROSCOPY – SOME HISTORY - SERS

SERS ENHANCEMENT



Dr. Martin Fleischmann
(1927 – 2012)

In 1973, M. Fleischmann et. al. observed very strong (x 10^6) Raman signals from pyridine adsorbed onto an electrochemically roughened Ag-electrode, initially associated to an increased surface area, published 1974. – **SERS EFFECT**

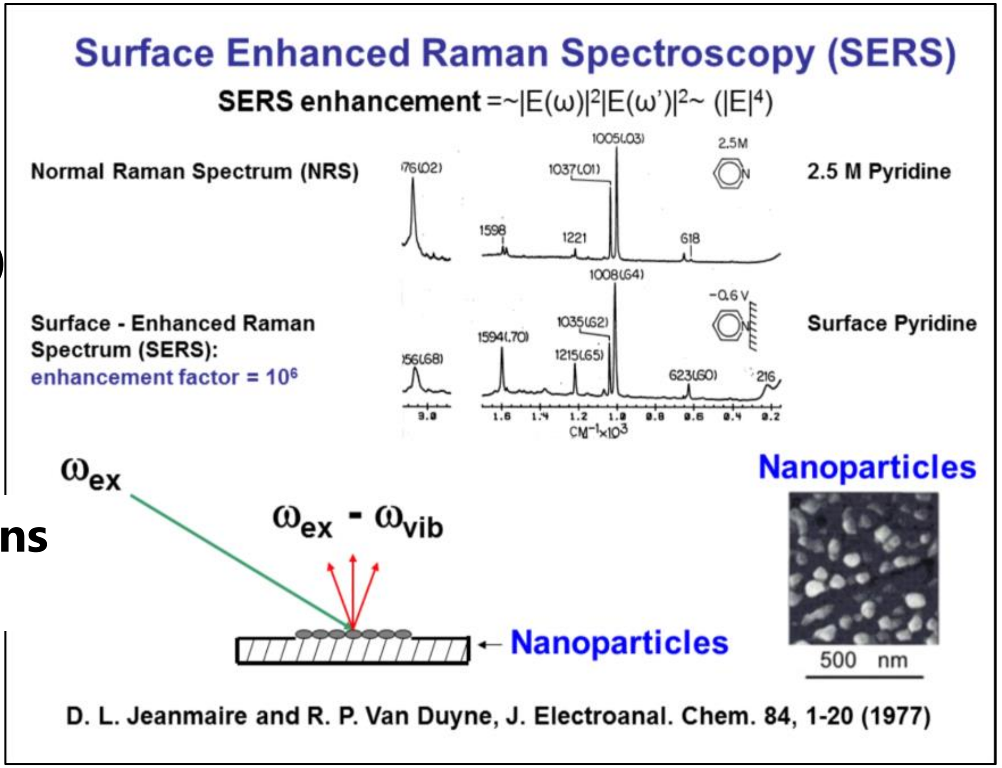
In 1977, **ENHANCEMENT NOT DUE TO SURFACE**

Jeanmaire and Van Duyne
electromagnetic effect (EM)

Albrecht and Creighton
electrochemical effect (CM)

R. Ritchie '57 **surface plasmons**
90's nanoplasmonics

201x, Prof. M. Moskovits,
plasmonic hot spots,



BASICS OF SPECTROSCOPY – SYNOPSIS

Raman spectroscopy is an analytical method requiring **minimal sampling** (like NIR) and revealing **sharp spectral peaks** as observed in MIR

Water is a «poor» Raman scatterer, making Raman spectroscopy an excellent method of analysis for samples in **aqueous solutions or wet environment.**

Non-polar molecules are strong Raman scatterer.

Weak scattering signal. → SERS

Achille's heal remains the **sample fluorescence**

CHALLENGES AT SERS:

- **QUANTITATIVE MEASUREMENT**
- **REPRODUCIBILITY**
- **CONTAMINATION**

OUTLINE

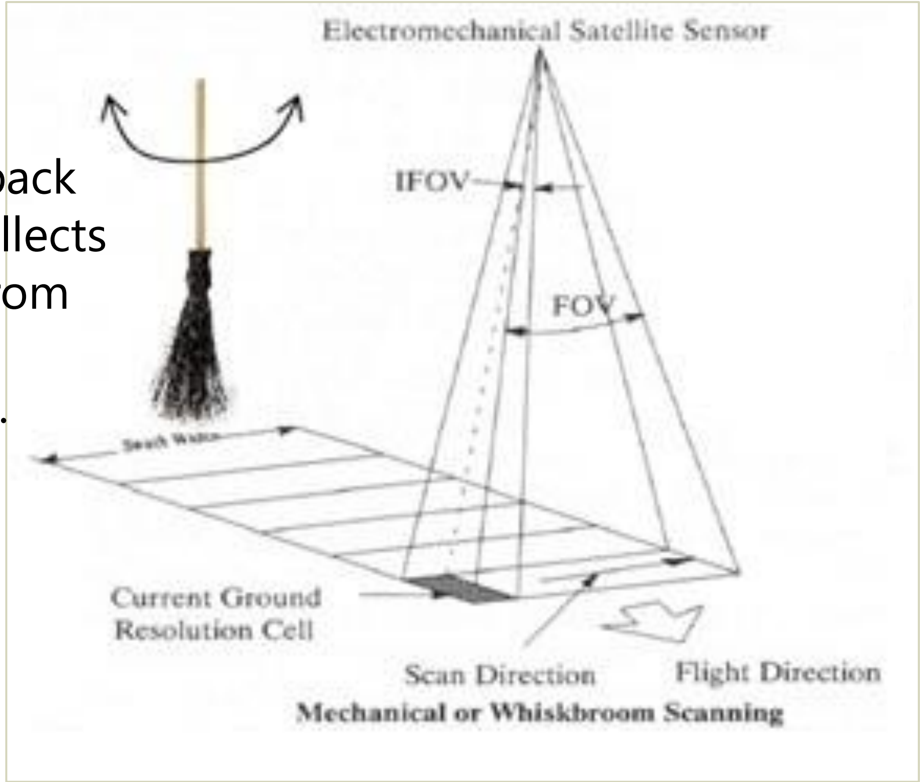
- INTRODUCTION AiDEXA
- BRIEF CASES DESCRIPTION
- BASICS OF SPECTROSCOPY
- **BUILDING BLOCKS OF OPTICAL SPECTROSCOPY INSTRUMENTATION**
- CONCLUSION, RECOMMENDED LITERATURE

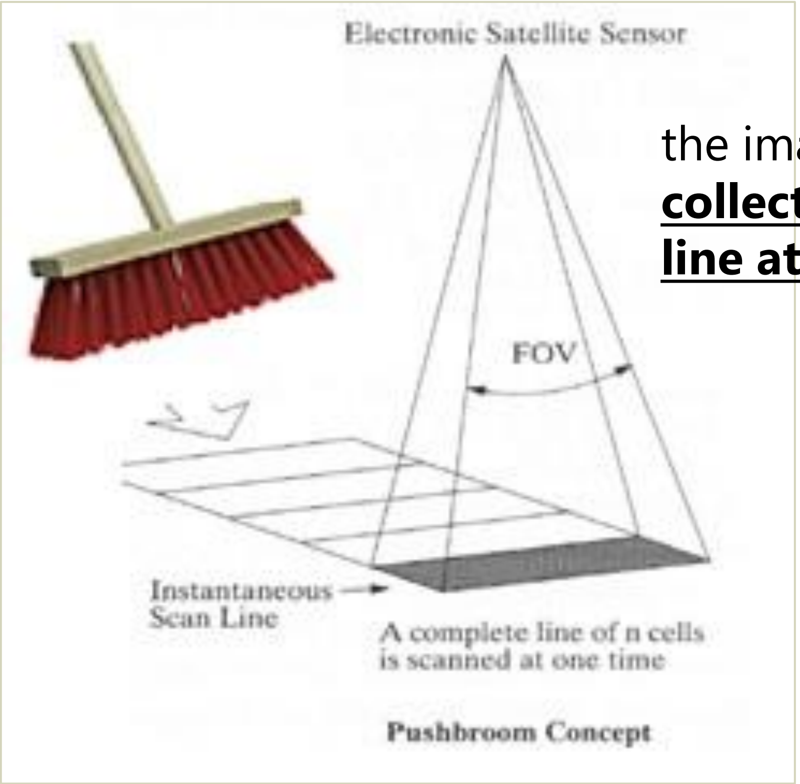
BUILDING BLOCKS: PUSHBROOM SCANNER

Whisk broom vs Push broom Scanner

as used in eRPHiX

a mirror moves back and forth and collects measurements from **one pixel in the image at a time**.





the image is **collected one line at a time**

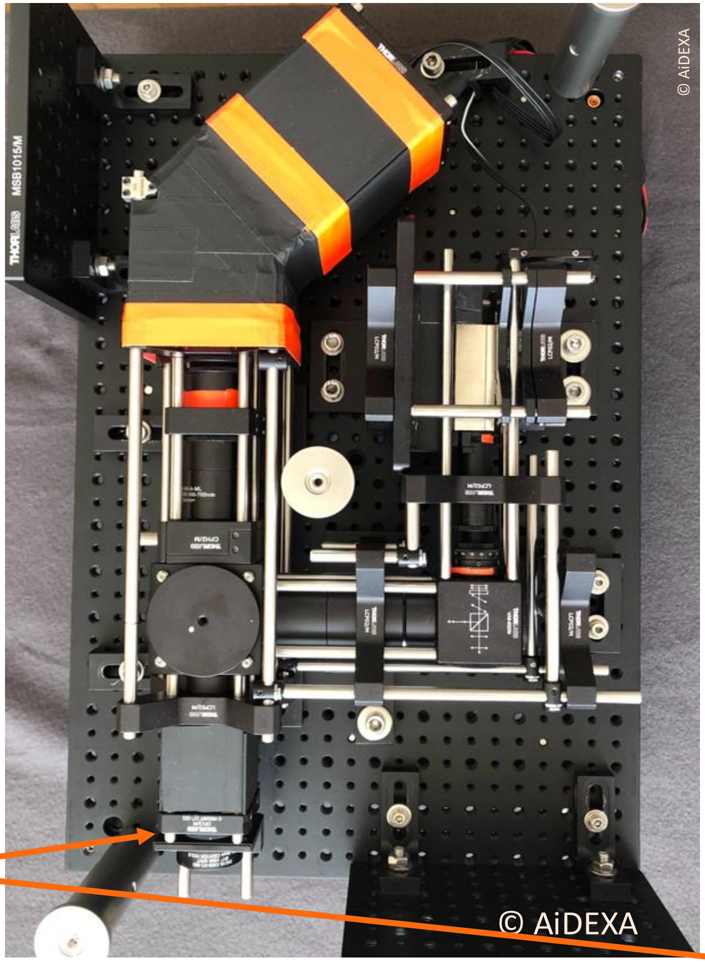
Source: *A whisk broom scanner sweeps in a direction perpendicular to the flight path, collecting one pixel at a time. A linear array detector advances with the spacecraft's motion, producing successive lines of image data (analogous to the forward sweep of a push broom). Images courtesy of Florian Hillen* https://www.l3harrisgeospatial.com/Support/Self-Help-Tools/Help-Articles/Help-Articles-Detail/ArtMID/10220/ArticleID/16262/Push-Broom-and-Whisk-Broom-Sensors

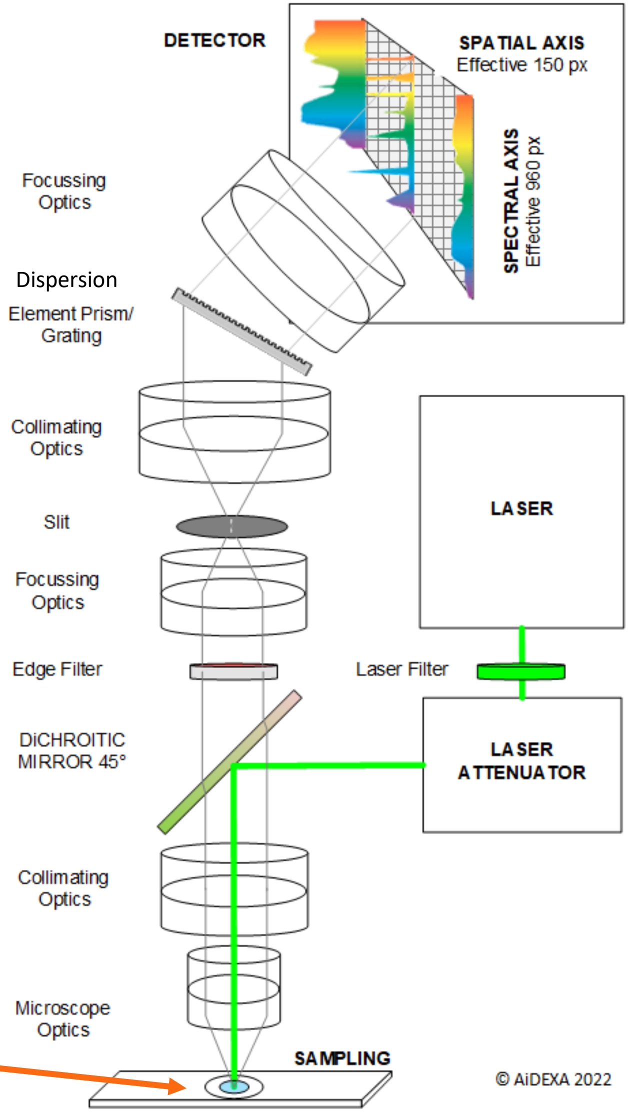
BUILDING BLOCKS OF SPECTROSCOPiC INSTRUMENTATiON

eRPHiX

experimental
Raman
Plasmonic
Hyperspectral
Imaging

eRPHiX is used to visualize a hyperspectral imaging cube, the continuous scan of a colloidal droplet or nano-substrate on a microscopy slide





BUILDING BLOCKS OF SPECTROSCOPiC INSTRUMENTATiON

- LiGHT: LASER EXCITATION SOURCE
- OPTICS: PHOTONIC DELIVERY & COLLECTION
- DISPERSION: SPECTROGRAPH
- DETECTORS: CCD to CMOS 2D FPA
- SOFTWARE: Hyperspectral imaging
- ENHANCEMENT: Colloids & Hard Substrates

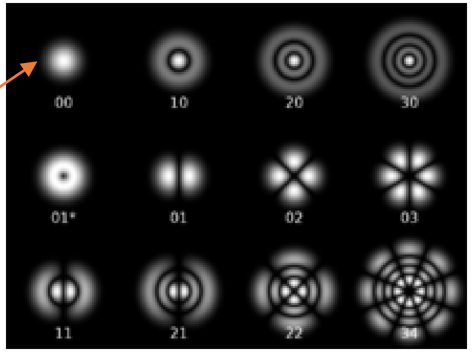
Optimization of each of these components into an integrated system is critically important.

Only an optimized system can be capable of measuring the <u>lowest concentration of species in the shortest time possible.</u>

BUILDING BLOCKS: LIGHT SOURCES

Lasers for Raman spectroscopy must show:
- Gaussian beam profile, TEM_{00} mode only
- High wavelength stability → temperature stabilization
- Narrow emission bandwidth, FWHM max. 1pm
- Absence of side lines → prevent mode hopping (stabilize case temperature and injection current, prevent optical feedback)

Only TEM_{00} mode used in Raman



Parameter	Minimum Value	Typical Value	Maximum Value
Central Wavelength, nm	531.9	532.0	532.1
Longitudinal modes	-	Single	-
Spectral line width FWHM, pm	-	0.2 [1]	1
Output power, mW	-	50 [2]	-
Side-mode suppression ratio (SMSR), dB	40	50	60
Power stability, % (RMS, 8 hrs)	-	0.4 [3]	1
Power stability, % (peak-to-peak, 8 hrs)	-	2 [4]	3
Noise, % (RMS, 20 Hz to 20 MHz)	-	0.5 [5]	1

Source: https://integratedoptics.com/cw-lasers/532-nm-lasers/532-nm-slm-laser-dpss;-free-space

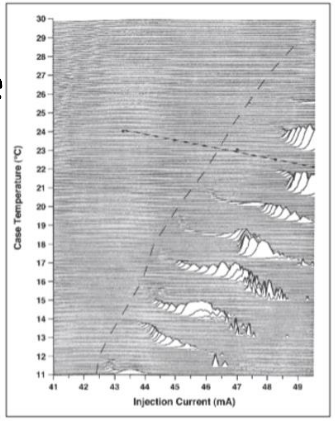
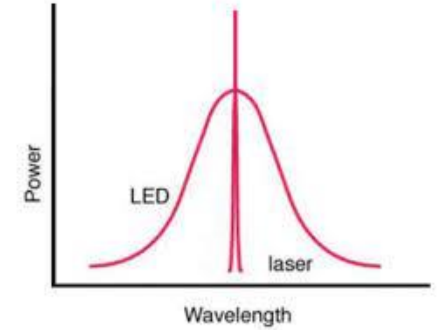


Figure 7. Laser Stability Map. Plots of ac voltage vs. current taken at different temperatures are plotted in an

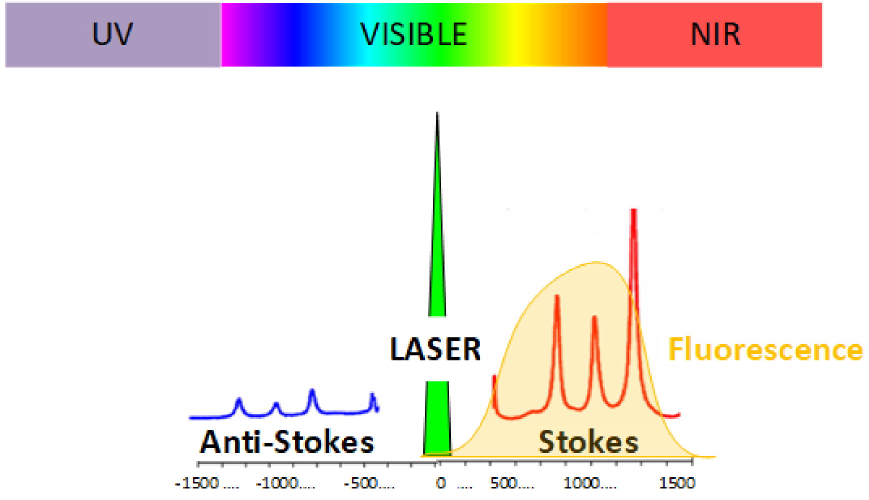


Lambertian LUXEON Rebel at Test Current [1], .

Table 2.

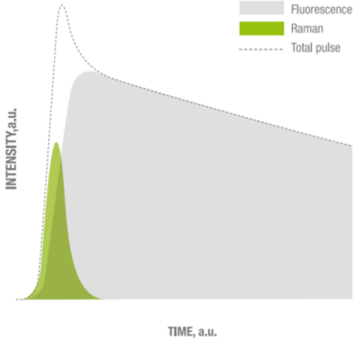
Color	Dominant Wavelength (nm)			Typical Spectral Half-width [3] (nm) $\Delta\lambda_{1/2}$
	Minimum	Typical	Maximum	
Red-Orange	613.0 nm	617.0 nm	620.0 nm	20
Amber	587.0 nm	590.0 nm	592.0 nm	14

BUILDING BLOCKS: FLUORESCENCE MITIGATION DESIGNS



Other fluorescence mitigation designs:

TimeGated, pulsed laser, Raman fast vs Fluorescence has longer decay time
https://www.timegate.com/products



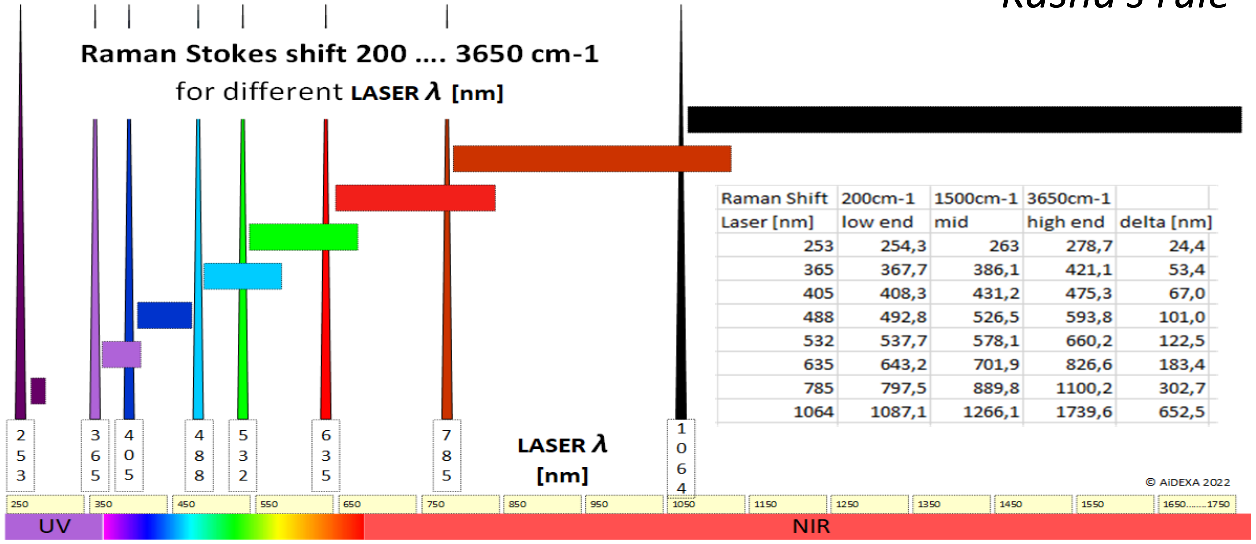
Bruker - Sequentially Shifted Excitation SSE ™ US patent 8,570,507B1 - temperature tuned diode lasers. *Raman shifts as function of excitation vs. independent fluorescence emission.*

Kasha's rule

To minimize laser-induced-fluorescence:

Select excitation laser λ VIS-NIR / UV:
tune the laser wavelength where the probability of interference from the fluorescence signal is minimal.

$$\lambda[nm] = \frac{1}{\frac{1}{\lambda_{ex}[nm]} - \frac{Raman\ \ shift[cm^{-1}]}{10^7}}$$



Raman Shift	200cm-1	1500cm-1	3650cm-1	
Laser [nm]	low end	mid	high end	delta [nm]
253	254,3	263	278,7	24,4
365	367,7	386,1	421,1	53,4
405	408,3	431,2	475,3	67,0
488	492,8	526,5	593,8	101,0
532	537,7	578,1	660,2	122,5
635	643,2	701,9	826,6	183,4
785	797,5	889,8	1100,2	302,7
1064	1087,1	1266,1	1739,6	652,5

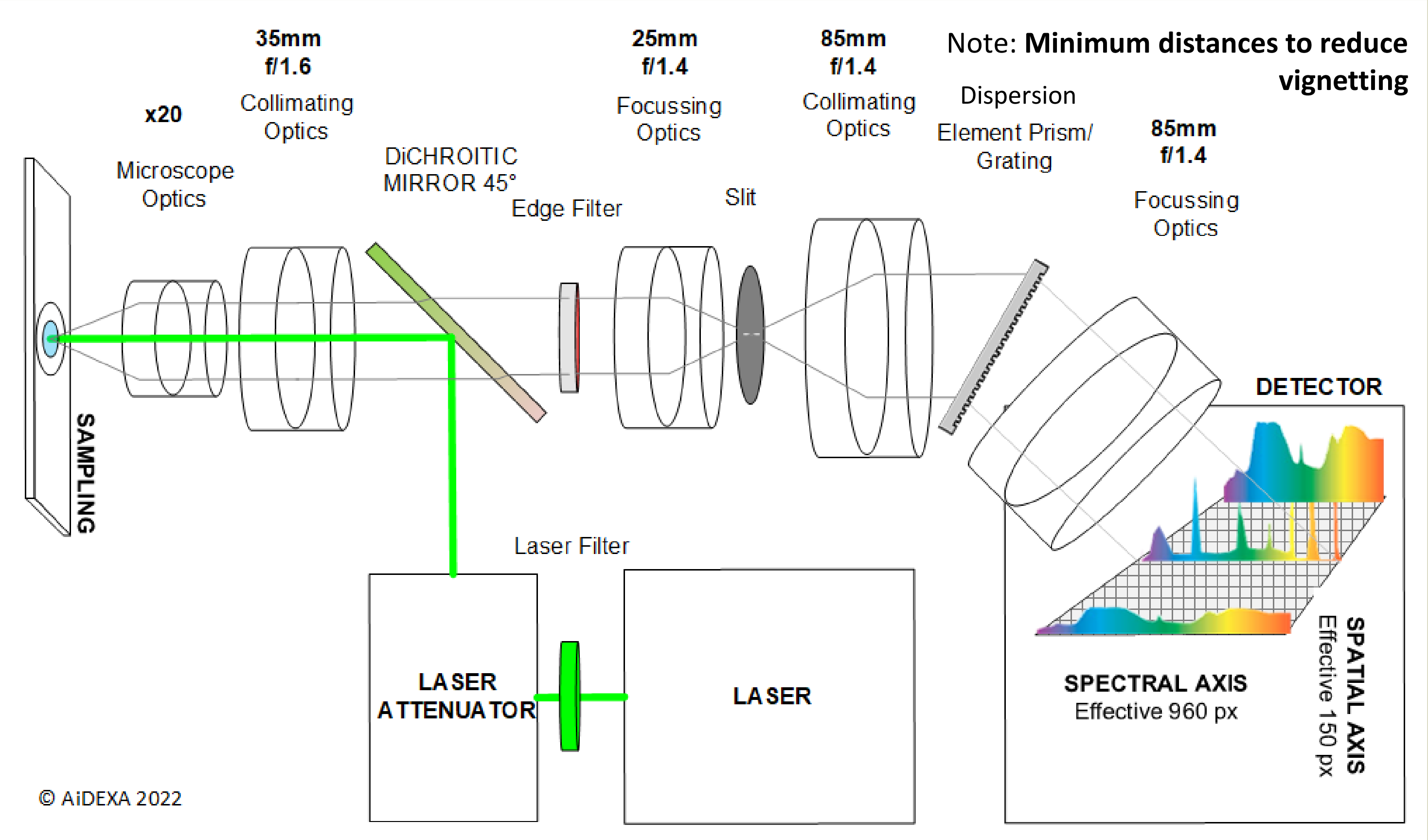
https://www.researchgate.net/profile/R-Hochleitner/publication/41625128_Raman_SpectroscopyA_Powerful_Tool_for_in_situ_Planetary_Science/

BUILDING BLOCKS: OPTICS DELIVERY & COLLECTION

OPTICS: PHOTONIC DELIVERY & COLLECTION

*„Traditional Raman instruments lose **80-97%** of the photons"* in Rabus, Optofluidics, 2019, pg. 354

Optimum coupling design has to ensure a constant flux of photons from light source to sample and from sample to spectrograph to detector with a minimal loss.



BUILDING BLOCKS: OPTICS - SPECTROGRAPH

1785 — Gratings Hairs !

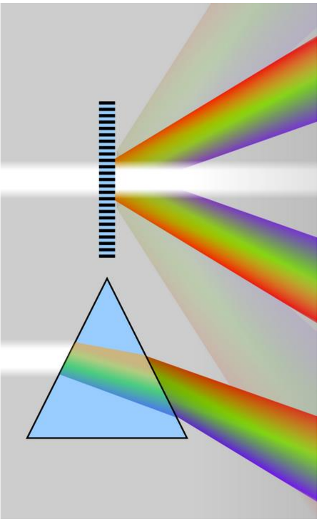
1928 — prisms → Refraction based: n = f(λ)

1953 — ruled precision gratings

1972 — holographic gratings

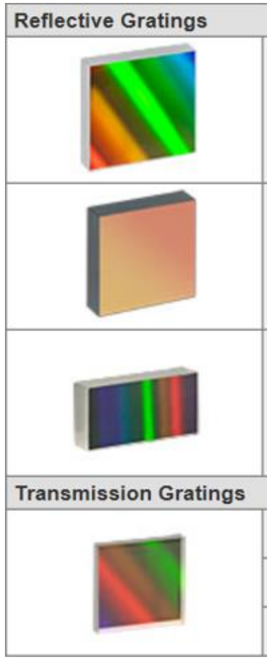
Diffraction based

https://en.wikipedia.org/wiki/Prism



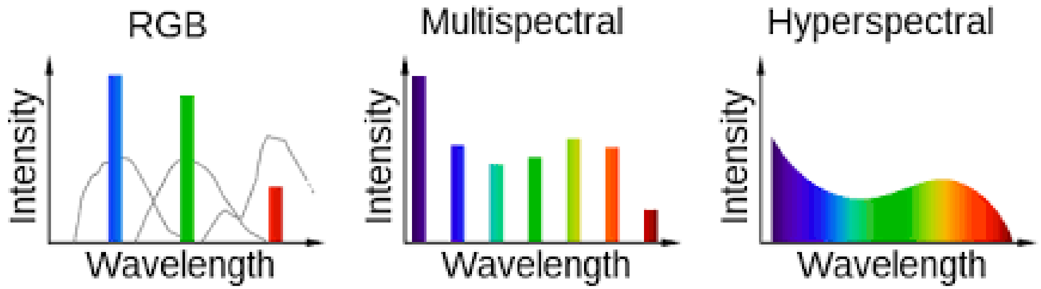
+1 dif. order

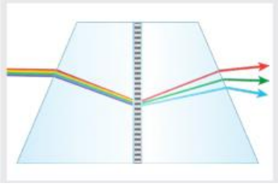
-1 dif. order



https://www.thorlabs.com/

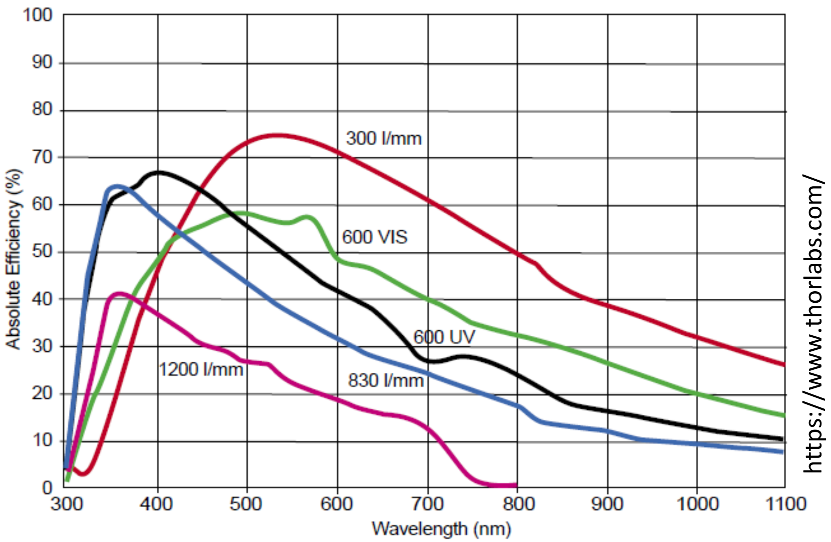
Go for Hyperspectral: Sufficient spectral resolution to resolve Raman peaks and to enable derivative spectroscopy





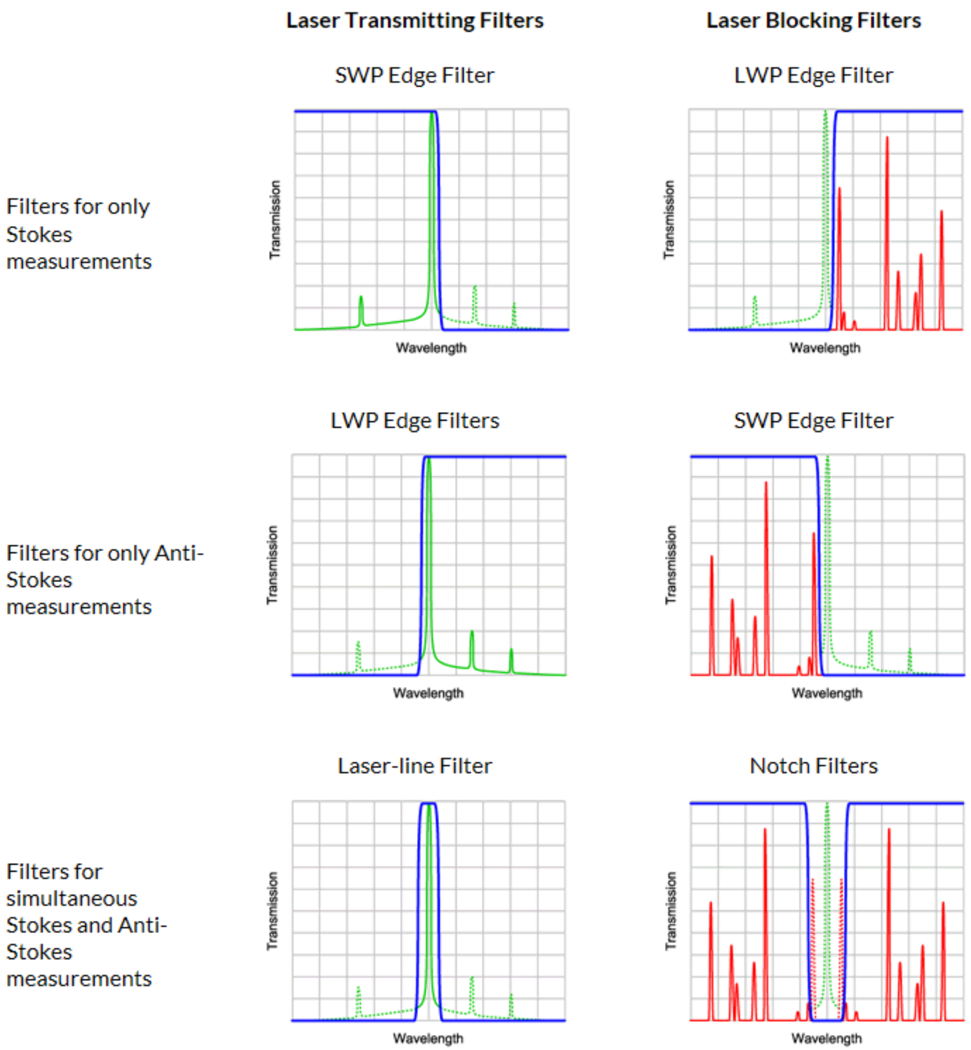
PGP or Grisms

Highest flexibility with Volume Phase Holographic (VPH) transmission gratings



https://www.thorlabs.com/

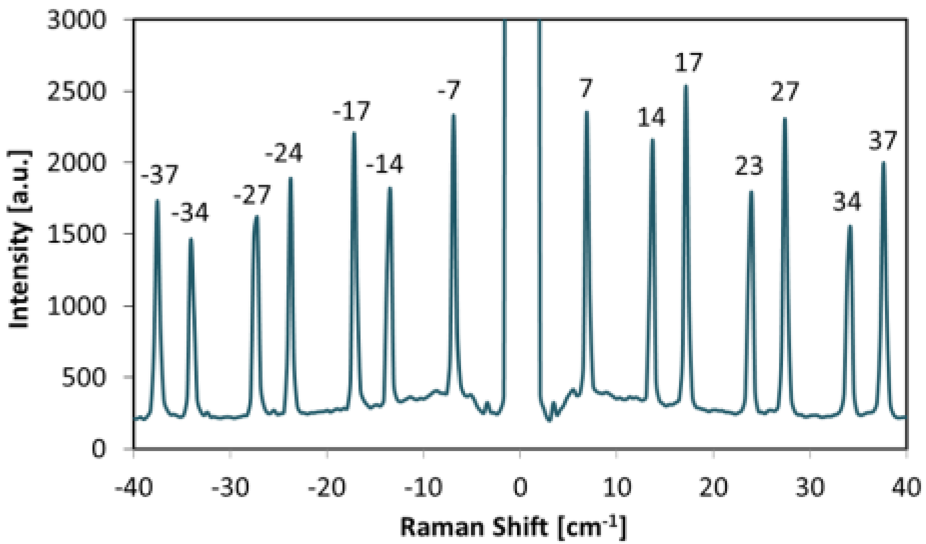
BUILDING BLOCKS: OPTICS - FILTERS



https://www.semrock.com/filter-types-for-raman-spectroscopy-applications.aspx

Relative novelty

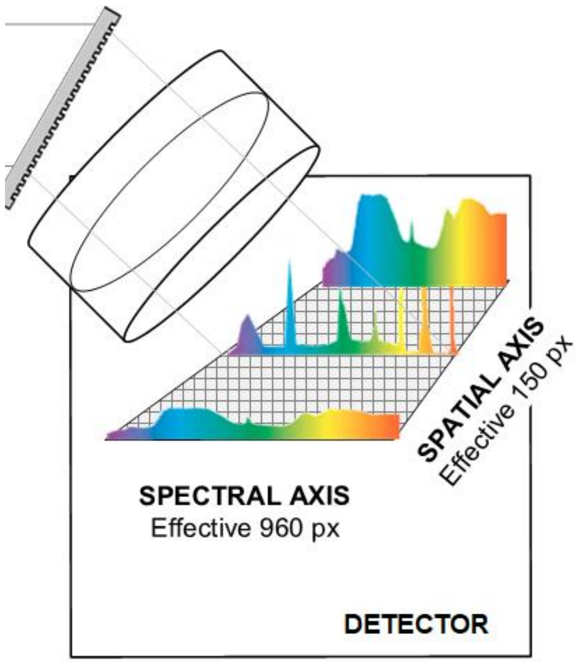
Volume Bragg Gratings for Ultra-Low Frequency Raman Spectroscopy



Extend Raman system into the THz frequency range (5-200 cm^{-1}) for crystal lattice modes features

https://www.optigrate.com/Raman/2020%20Raman%20Thomson%20notch%20filters%20web.pdf

BUILDING BLOCKS: DETECTORS – FOCAL PLANE ARRAYS

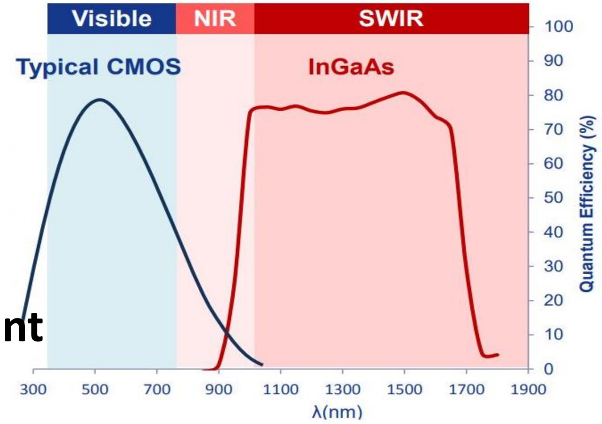


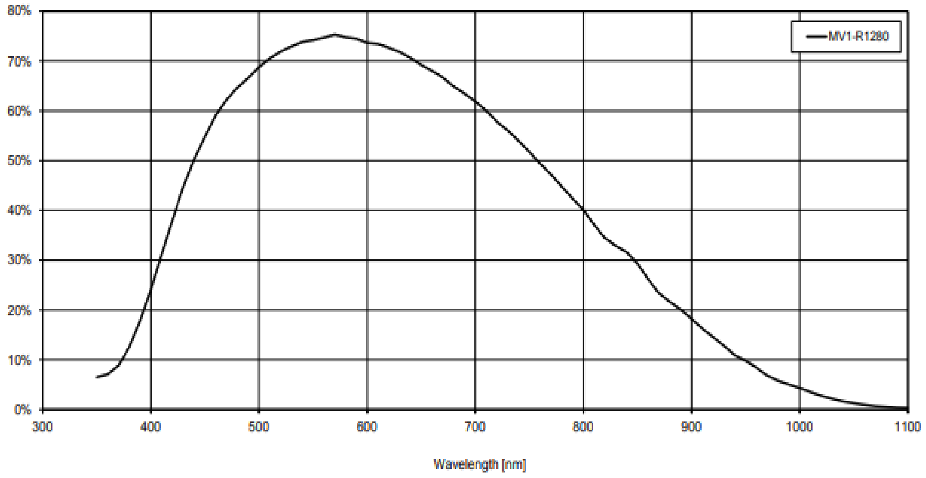
choose as a function of the laser excitation source + hyperspectral resolution

high QE,
low dark current,
low read-out noise

QE: % of photons converted to electrons at a specific wavelength by the sensor.

Indicator for low light sensitivity. QE > 70%





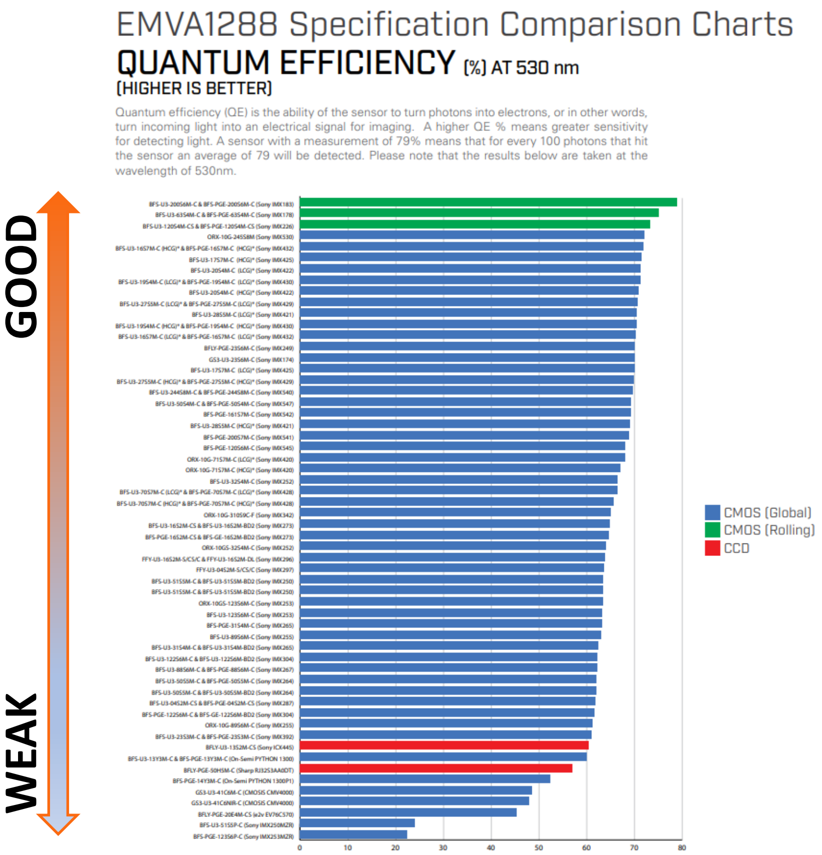
In VIS-range **BSI CMOS is the dominant technology for Hi-Perf. FPA sensors.**

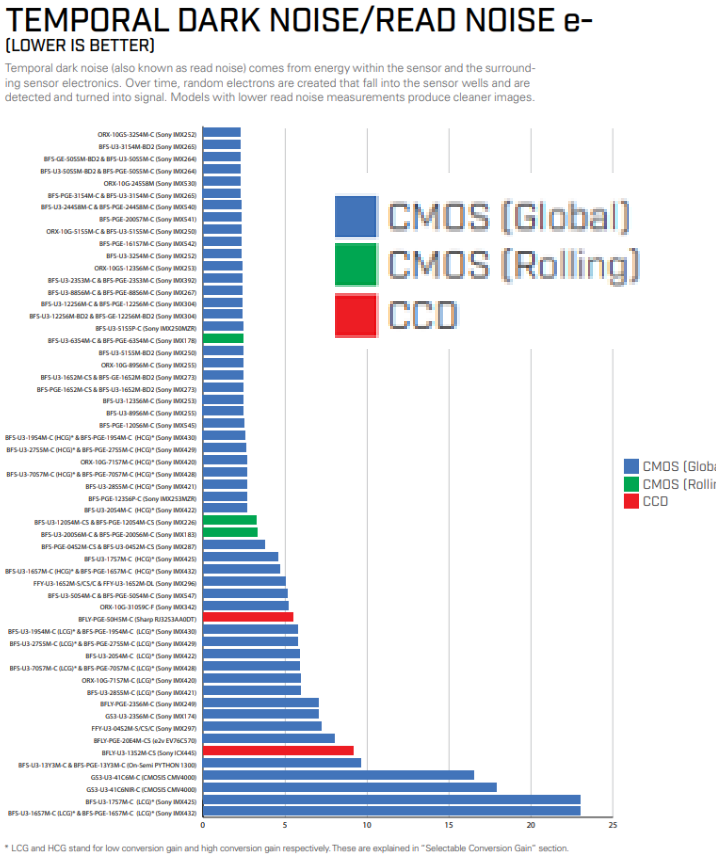
Note: SONY announced the closure of their CCD manufacturing in 2015.

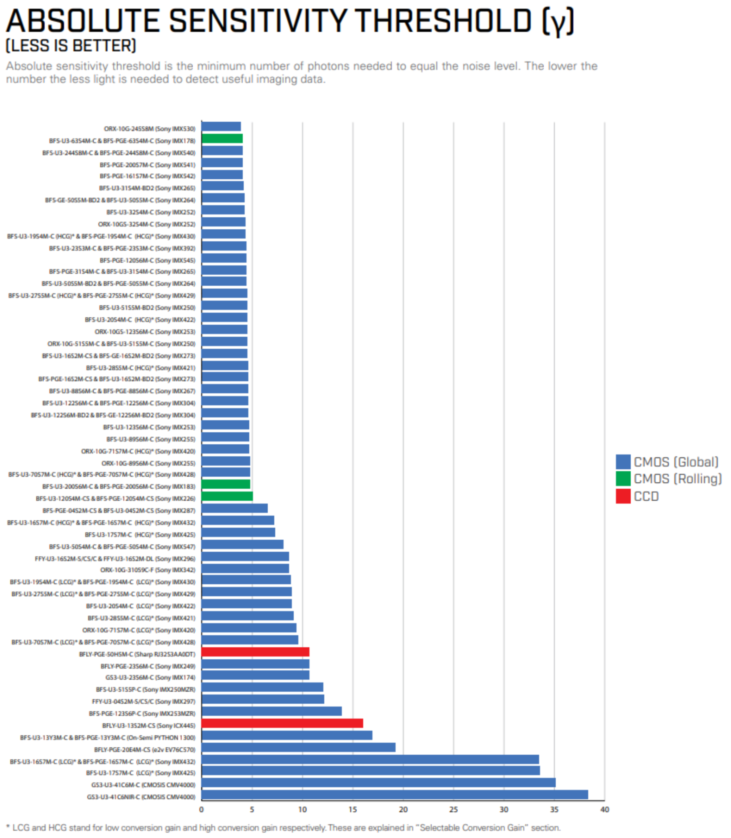
Source: Sebastien Frasse-Sombet @ Vision Days 2018, http://image-sensors-world.blogspot.com/2019/01/sofradir-uncooled-ingaas-imagers.html

Quantum efficiency curve of the monochrome ultra-low light sensor of the MV1-R1280 camera. Source: Photonfocus User Manual MAN066_e_V1_1_MV1_R1280_G2.pdf

BUILDING BLOCKS: DETECTORS – FOCAL PLANE ARRAYS







QE: % of photons converted to electrons at a <u>specific wavelength</u> by the sensor. Indicator for low light sensitivity. > 70%

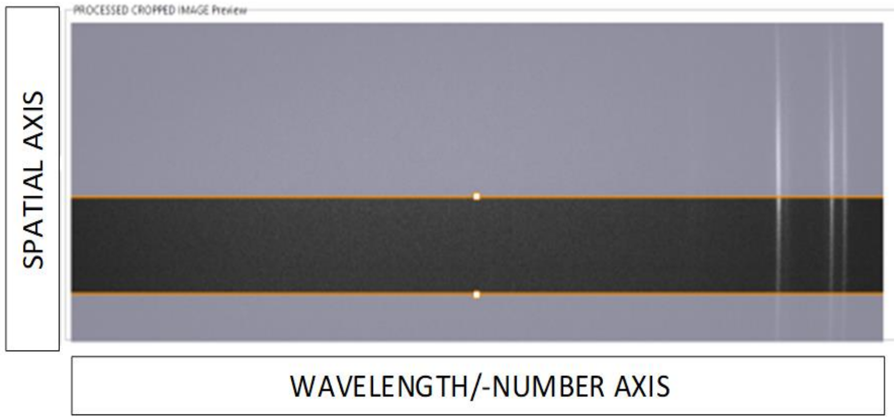
DARK NOISE: Noise in the sensor when there is no light. f(T). Higher dark noise creates grainier images. < 1e-

AST: Lowest intensity signal which can be detected above noise floor of sensor.

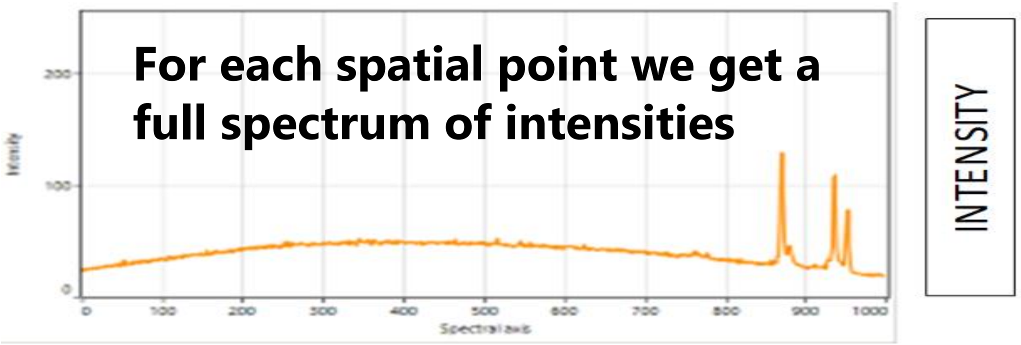
Source: Machine Vision – Area Scan Cameras | Teledyne FLIR FLIR 2022_MonoSensorReview_EN_Web.pdf

BUILDING BLOCKS: DETECTORS – FOCAL PLANE ARRAYS

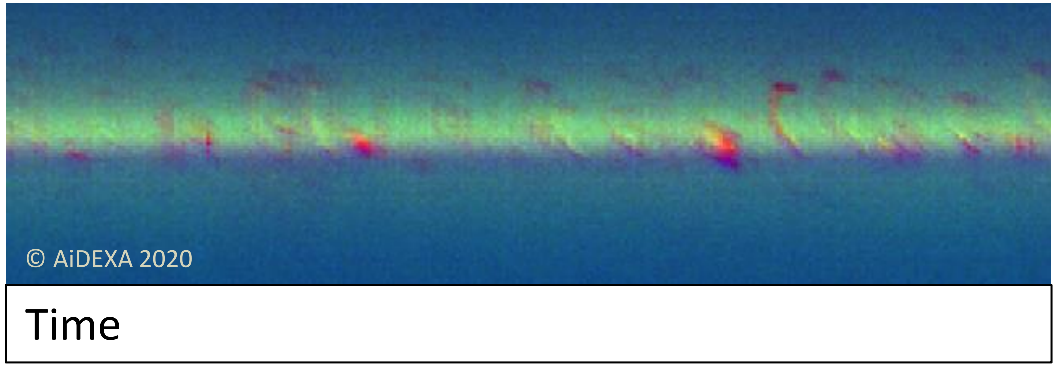
(Non) Linear dispersion of the different Raman intensities along the CMOS 2D array length resulting from the angular dispersion produced by the grating/prism



For each spatial point we get a full spectrum of intensities



As each line of points is scanned cyclically, we get a hyperspectral imaging cube.



With some data processing we can **VISUALIZE IN FALSE COLORS THE SPECTRAL CHANGES IN THE SCANNED LINE**.

This can be used to evaluate the **quality of SERS substrates** and **ultimately to develop novel applications.**

BUILDING BLOCKS: SOFTWARE HYPERSPECTRAL IMAGING

HYPERSPECTRAL IMAGING

PRE-PROCESSING DATA REGISTRATION

- SMILE, KEYSTONE COrr.
- DEFECT PIXELs Corr.
- WAVELENGTH CALIBRATION
- NOISE REDUCTION
- INTENSITY CALIBRATION

BASIC PROCESSING

- ABS. SPECTRA, SMOOTHING
- BASELINE CORRECTION
- NORMING: Relative spectra
- DIFFERENTIATION: 1st, 2nd
- DIFF. with NORMING

ROIs/ BINNING

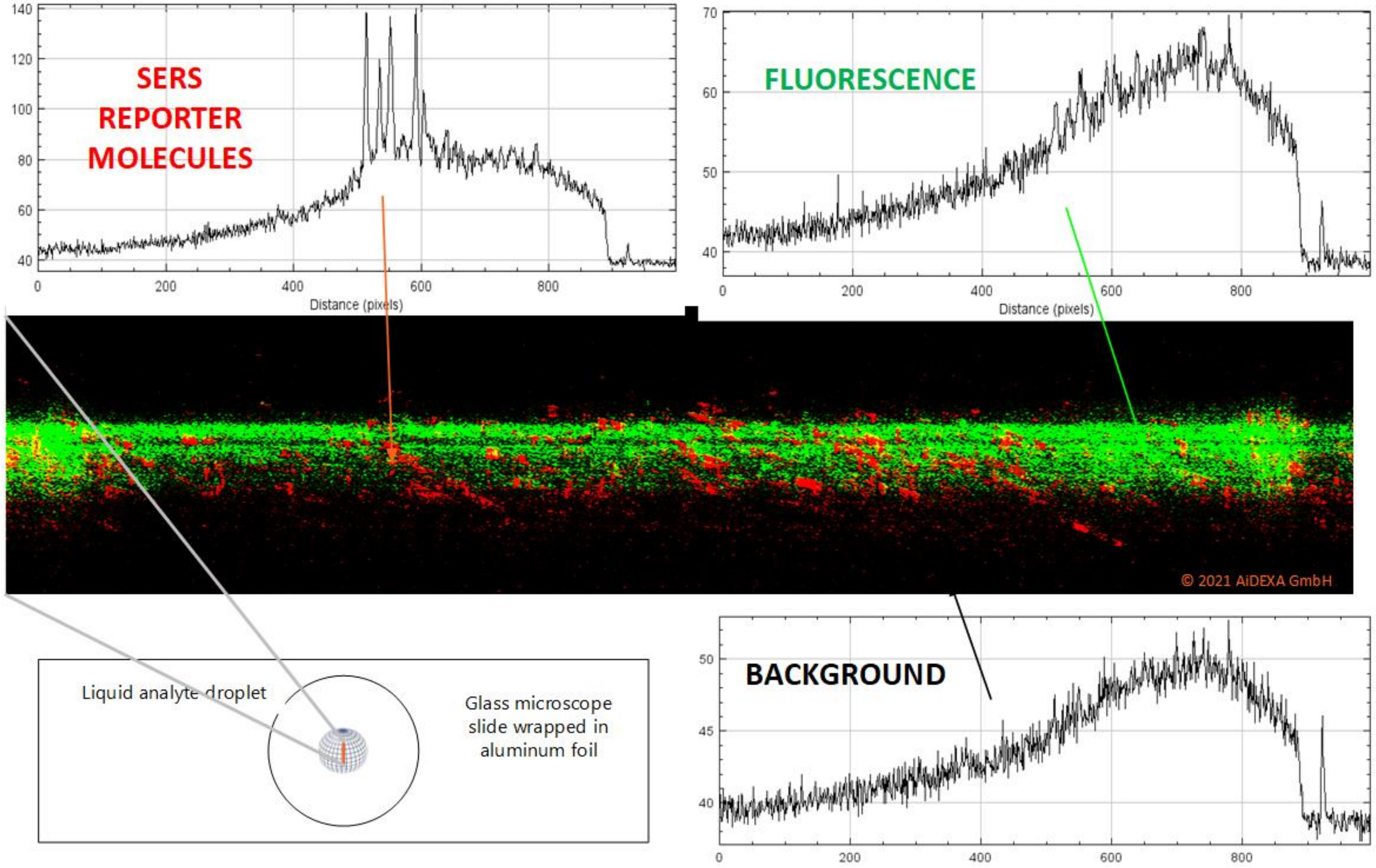
- MULTIPLE ROIs
- CONFIG. BINNING

FEATURE EXTRACTION

- Spectral Intensity, Area, Ratios
- MVA Chemometrics
- Classification and Regression
- Arithmetical functions
- Etc.......

FEATURE to FALSE COLOUR IMAGING

DERIVATIVE SPECTROSCOPY !



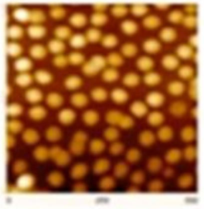
False colour visualization of 4 dimensional hyperspectral imaging cube.
Vertical section line of 1.5mm through colloidal droplet of 10-8M R6G in AuNP placed on aluminum foil covered glass slide and scanned with 10 fps (time is on the horizontal axis). Green visualizes spot of increased fluorescence; orange are hyperspectral pixels with SERS spectra of R6G.

BUILDING BLOCKS: ENHANCEMENT

Commercial SERS substrates

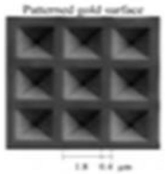
Solution-based

Colloids 10-100nm
AuNP, **AgNP**

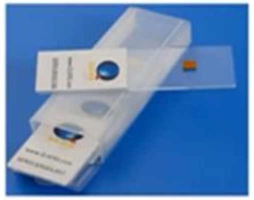


add 4 to 6M NaCl in H_2O, "the more the better"

Substrate-based



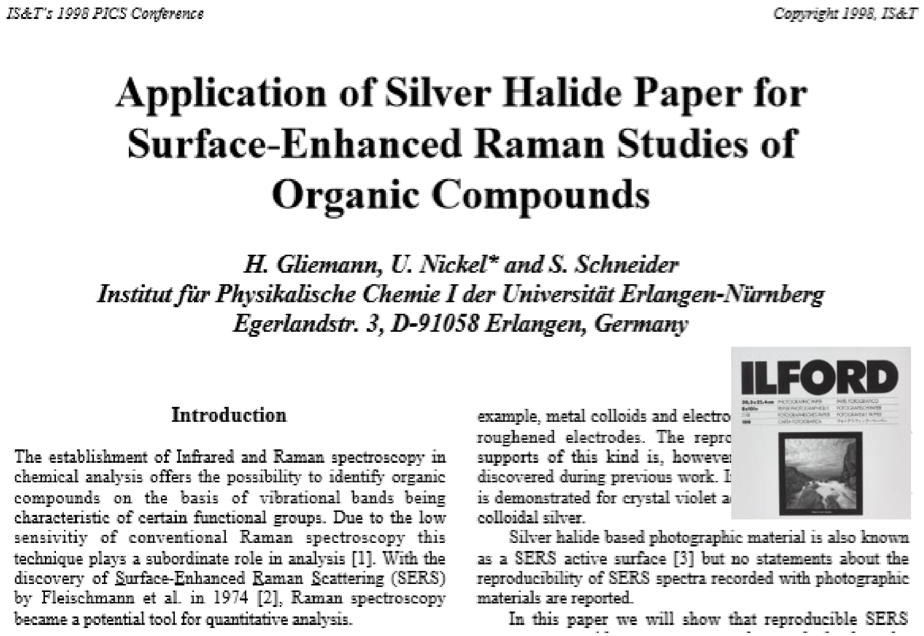
Klarite ™ Au-SERS



Q-SERS
(Nanova Inc.)

DiY SERS substrates

Leopold N, Lendl B. A New Method for Fast Preparation of Highly Surface-Enhanced Raman Scattering (SERS) Active Silver Colloids at Room Temperature by Reduction of Silver Nitrate with Hydroxylamine Hydrochloride. J Phys Chem B. 1072003; :57235727



IS&T's 1998 PICS Conference *Copyright 1998, IS&T*

Application of Silver Halide Paper for Surface-Enhanced Raman Studies of Organic Compounds

H. Gliemann, U. Nickel and S. Schneider*
Institut für Physikalische Chemie I der Universität Erlangen-Nürnberg
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Introduction

The establishment of Infrared and Raman spectroscopy in chemical analysis offers the possibility to identify organic compounds on the basis of vibrational bands being characteristic of certain functional groups. Due to the low sensitivitiy of conventional Raman spectroscopy this technique plays a subordinate role in analysis [1]. With the discovery of Surface-Enhanced Raman Scattering (SERS) by Fleischmann et al. in 1974 [2], Raman spectroscopy became a potential tool for quantitative analysis.

example, metal colloids and electro… roughened electrodes. The repro… supports of this kind is, howeve… discovered during previous work. I… is demonstrated for crystal violet a… colloidal silver.

Silver halide based photographic material is also known as a SERS active surface [3] but no statements about the reproducibility of SERS spectra recorded with photographic materials are reported.

In this paper we will show that reproducible SERS

CONCLUSION

- There are plenty of opportunities for value-adding contributions arising in Photonics in general and in particular hyperspectral imaging instrumentation

- Want to get measurement in-house?

Build a cheap fluorescence setup and explore excitation wavelengths before investing in a Raman system

Handheld Raman

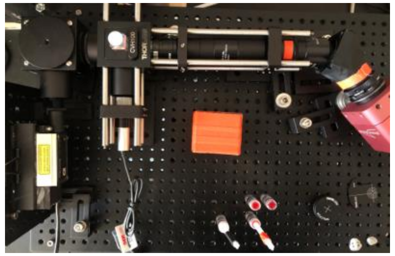
Very integrated
Not flexible enough

Research Grade Confocal Raman uScope

Very expensive
Req. skilled operator

Fiber Coupled Raman uScope

Flexible and
less expensive



Home brew experimental

Most flexible,
Enables learning

Make your choice and adapt as you move

RECOMMENDED LITERATURE

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Contact :

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Bundesministerium
Klimaschutz, Umwelt,
Energie, Mobilität,
Innovation und Technologie

FFG
Forschung wirkt.

THANK YOU FOR YOUR ATTENTION

APPENDIX

BUILDING BLOCKS: MEANS of INCREASING PERCEPTION

Chemical Additives To Enhance Or Quench: SERS – NPs , FLUORESCENT DYEs

Optical Throughput Optimization

Software: hyperspectral imaging, derivative spectroscopy, ML/DL, Ai etc.

Surface Enhanced Raman Spectroscopy for Single Molecule Protein Detection

REPRODUCIBILTY

CALIBRATION

TRANSFERABILITY

nature

Explore content ⌄ About the journal ⌄ Publish with us ⌄ Subscribe

nature > news > article

NEWS | 26 July 2022

Could machine learning fuel a reproducibility crisis in science?

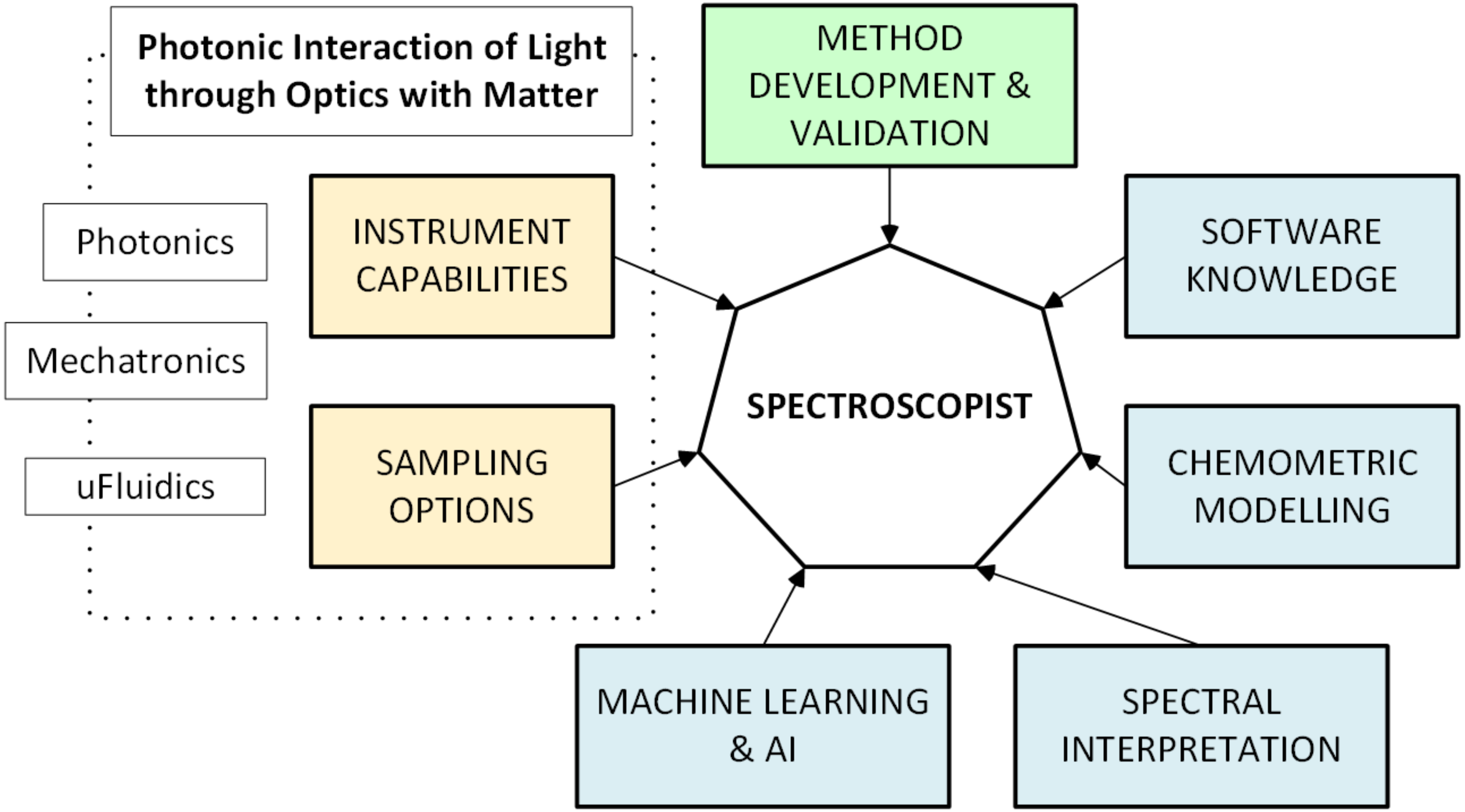
'Data leakage' threatens the reliability of machine-learning use across disciplines, researchers warn.

Elizabeth Gibney

Source: *https://doi.org/10.1038/d41586-022-02035-w*

Source: https://arxiv.org/pdf/2207.07048.pdf

BUILDING BLOCKS: SPECTROSCOPY SKILLS



Adapted by AiDEXA 2022, from R.D. McDowall, Spectroscopy Application Notebook, February 2010

BUILDING BLOCKS: MONOZUKURI & JUGAAD

Monozukuri,
excellence in making things

Japanese, the *monozukuri* spirit includes a sincere attitude towards production with pride, skill and dedication and **the pursuit of innovation and perfection.**

Jugaad,
frugal innovation

Hindi slang word, *jugaad* (say joo-gaardh). It means **"an improvisational style of innovation"** based on gut intelligence grown on experience. It doesn't have to be perfect or fancy; just good enough to satisfy immediate needs.

Foldscope: Origami-Based Paper Microscope, a 50-cent paper microscope that magnifies up to 2000 times

Liter of Light

https://www.huffpost.com/entry/plastic-bottles-electricity_n_596e64f4e4b0000eb1968bb5