HOW DO YOU VIEW?

Illuminating biological processes and structures through (bio)chemical detection.

Ever since Antonie van Leeuwenhoek first described the microbial world seen through his microscope, scientists have been looking for new ways to watch biology unfold. These days much of the imaging that goes on in a lab occurs beyond the oculars and takes advantage of sophisticated chemistry to "see" biological processes and structures. As we approach the 345th anniversary of van Leeuwenhoek's first publication, let's take a moment to celebrate these powerful techniques that have shed so much light on how life works.

Radiochemistry | Chemiluminescence | Fluorescence

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RADIOCHEMISTRY

Invaluable for their ability to deliver direct quantitation at the single-molecule level, radiolabeled chemicals were once the only method for following dynamic biochemical processes. However, radioisotope usage has gradually declined from a peak towards the end of the last century, as safer alternatives have been developed. Still, for some applications, the advantages of radiolabeled compounds outweigh the risks, which can be ameliorated through the use of safety procedures.

DID YOU KNOW? Radioisotope use for life science research became widespread in the 1950s in part due to efforts by the Atomic Energy Commission to promote peace-time uses for nuclear technology.[1]

APPLICATIONS Commonly used for: imaging tissue samples, Southern blots, northern blots, and kinase assays.

Isotope	Half-life	Decay Mode	Emission (MeV)	Shielding	Maximum Range in Air	Maximum Range in Tissue
^{3}H	12.3 y	β-	0.0186	None	0.25 in (0.6 cm)	Negligible
^{14}C	5730 y	β-	0.156	None	10.0 in (24 cm)	0.012 in (0.28 mm)
^{18}F	1.8 h	β+, EC	0.63 (β+), 1.66 (EC)	Plastic	62 in (1.6 m)	0.09 in (0.23 cm)
^{32}P	14.3 d	β-	1.71	Plastic	20 ft (6.1 m)	0.33 in (0.76 cm)
^{35}S	87.3 d	β-	0.17	None	10.2 in (26 cm)	0.015 in (0.32 mm)
^{125}I	59.5 d	EC, γ	0.19 (EC), 0.35 (γ)	Lead	N/A	N/A

DID YOU KNOW? Nuclear testing during the early Cold War period increased the amount of ^{3}H on the Earth's surface by several orders of magnitude, making ^{3}H a useful tracer for atmospheric patterns.[2]

1. A.N.H. Creager, *Life Atomic: A history of radioisotopes in science and medicine.* Chicago, IL. The University of Chicago Press, 2013. **2.** M.H. England and E. Maier-Reimer, "Using chemical tracers to assess ocean models," *Rev Geophys* 39(1): 29-70, 2001. **3.** P. Lorimier, et al., "Enhanced chemiluminescence: a high-sensitivity detection system for in situ hybridization and immunohistochemistry," *J Histochem Cytochem* 41(11): 1591-1597, 1993. **4.** P. Khan, et al., "Luminol-Based Chemiluminescent Signals: Clinical and Non-clinical Application and Pitfalls Sites," *Appl Biochem Biotechnol* 173(2): 333-355, 2014. **5.** G.H. Thorpe, "Phenols as enhancers of the chemiluminescent horseradish peroxidase-luminol-hydrogen peroxide reaction: application in luminescence-monitored enzyme immunoassays," *Clin Chem* 31(8):1335-1341, 1985. **6.** G.G. Stokes, "XXX. On the change of refrangibility of light," *Phil Trans R Soc Lond* 142:463-562, 1852. **7.** A. Shirbet and Govindjee, "On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: basics and applications of the OJIP fluorescence transient," *J Photochem Photobiol B* 104(1-2):236-257, 2011. **8.** L. Yuan, et al., "Far-red to near infrared analyte-responsive fluorescent probes based on organic fluorophore platforms for fluorescence imaging," *Chem Soc Rev* 42(2):622-661, 2013. **9.** D.R. Tsai and M.R. Hamblin, "Biological effects and medical applications of infrared radiation," *J Photochem Photobiol B* 170:197-207, 2017. **10.** M.V. Marshall, et al., "Near-Infrared Fluorescence Imaging in Humans with Indocyanine Green: A Review and Update," *Open Surg Oncol J* 2(2): 12-25, 2010. **11.** E.A. Owens, et al., "Tissue-Specific Near-Infrared Fluorescence Imaging," *Acc Chem Res* 49(9):1731-1740, 2016.

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CHEMILUMINESCENCE

Highly specific, extremely sensitive, safer than radioisotope imaging, and more amenable to signal amplification than fluorophores,[3] chemiluminescent assays such as ELISAs and Western blots are almost ubiquitous in life science laboratories. However, because chemiluminescence harnesses biological reactions, its reproducibility is affected by experimental and environmental factors such as reaction duration, reactant amount, light exposure, and environmental conditions (*e.g.*, temperature, buffers).[2]

APPLICATIONS Commonly used for: ELISAs, Western blots.

DID YOU KNOW? The first recorded chemiluminescent reaction was performed in 1669 by the German alchemist Hennig Brand, who was trying to produce gold from phosphorus, which he distilled from urine.[2]

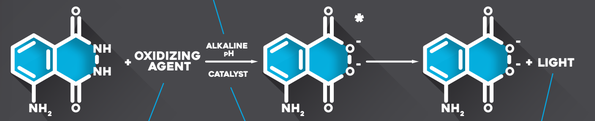
The Enzyme-Luminol Reaction

Simple, selective, and effective, luminol is arguably the most well-known chemiluminescent dye[4]

Luminol generates chemiluminescence when the dianion form is oxidized, creating excited intermediates which produce light during stabilization to the ground state

Complexities Apart from the oxidizer and catalyst, chemiluminescence intensity is also affected by experimental conditions such as pH (alkaline is best – but not too alkaline), heat (luminol is thermally unstable), luminol concentration, and protein concentration[4]

ECL Phenols and other compounds can enhance the magnitude and stability of catalyzed luminol oxidation-derived light emission by as much as 1,000-fold,[5] leading to the advent of enhanced chemiluminescence (ECL)



Oxidizers Commonly used oxidizing agents include ozone, halogens, singlet oxygen, hydrogen peroxide, and hypochlorites. Of these, hydrogen peroxide is most popular because it augments the luminescent intensity of luminol.[4]

Enzymes, including horseradish peroxidase (HRP) and alkaline phosphatase (Alk Phos), as well as metal ions serve as catalysts for the luminol-oxidant reaction

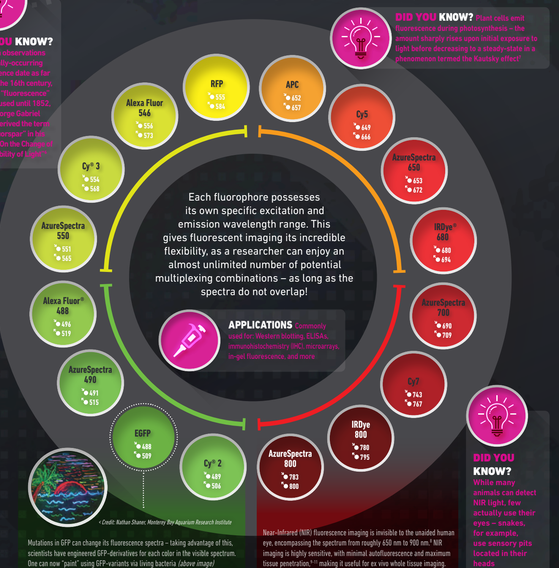
Detection The emission range of luminol is typically ~370-490 nm with a maximum at ~420 nm, but can vary significantly. For example, the presence of iron has been documented to cause a spectrum shift resulting in a maximum emission of 455 nm.[4]

FLUORESCENCE

Often associated with microscopy, fluorescence-based techniques have been rapidly expanding from the microscope slide and into the heart of the lab. Another alternative to radioactivity, non-microscopy uses for fluorescence became more widespread in part as a result of the human genome sequencing project and the development of dye-based sequencing. From there, rapid advances in detector technology and powerful computer processors have facilitated fluorescence use for protein detection, such as in Western blots, where the ability to use several fluorophores at once (multiplexing) can greatly enhance experimental efficiency.

DID YOU KNOW? Although observations of naturally-occurring fluorescence date as far back as the 16th century, the term "fluorescence" was not used until 1852, when George Gabriel Stokes derived the term from "fluorspar" in his treatise "On the Change of Refrangibility of Light".[6]

DID YOU KNOW? Plant cells emit fluorescence during photosynthesis – the amount sharply rises upon initial exposure to light before decreasing to a steady-state in a phenomenon termed the Kautsky effect.[7]



Credit: Nathan Shaner, Monterey Bay Aquarium Research Institute

Mutations in GFP can change its fluorescence spectra – taking advantage of this, scientists have engineered GFP-derivatives for each color in the visible spectrum. One can now "paint" using GFP-variants via living bacteria *(above image)*

Near-Infrared (NIR) fluorescence imaging is invisible to the unaided human eye, encompassing the spectrum from roughly 650 nm to 900 nm.[8] NIR imaging is highly sensitive, with minimal autofluorescence and maximum tissue penetration,[9,10] making it useful for ex vivo whole tissue imaging.

DID YOU KNOW? While many animals can detect NIR light, few actually use their eyes – snakes, for example, use sensory pits located in their heads

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