先週のクイズの答え

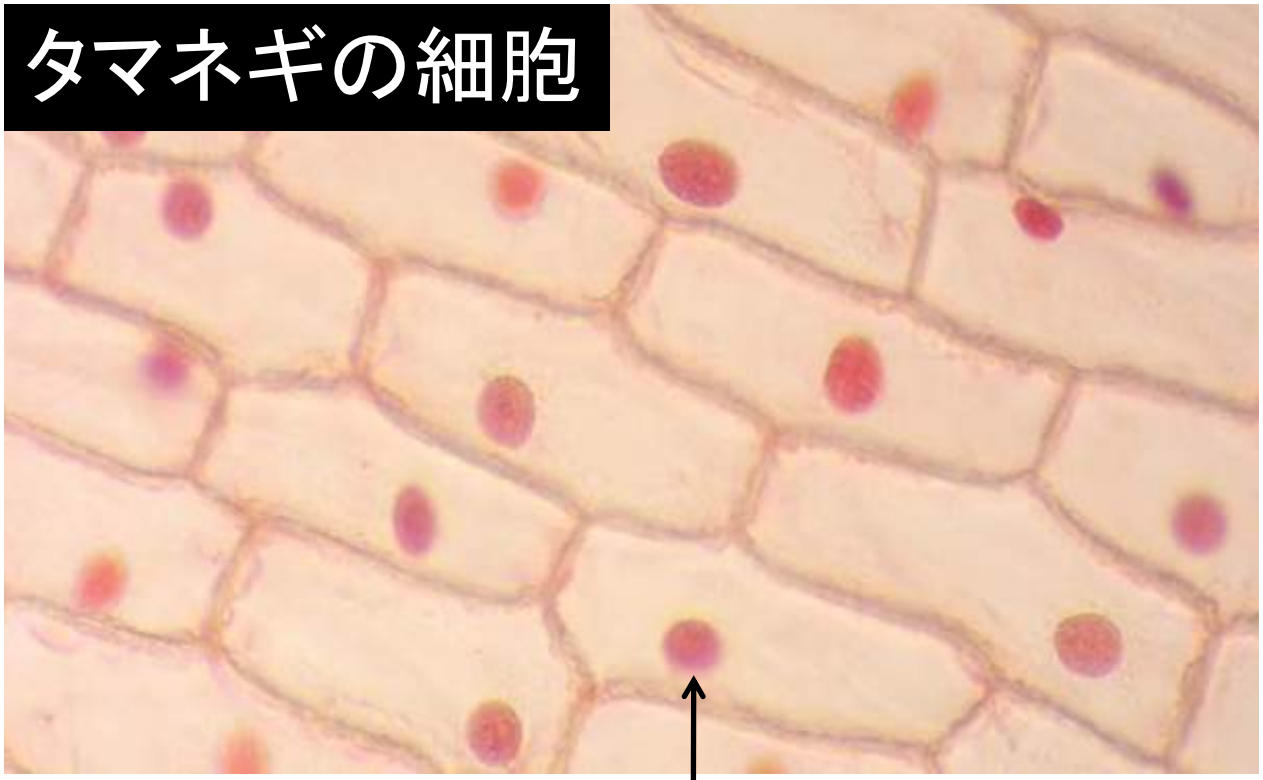
バイオイメージングとは？

Bioimaging

生物（個体、組織、細胞など）の

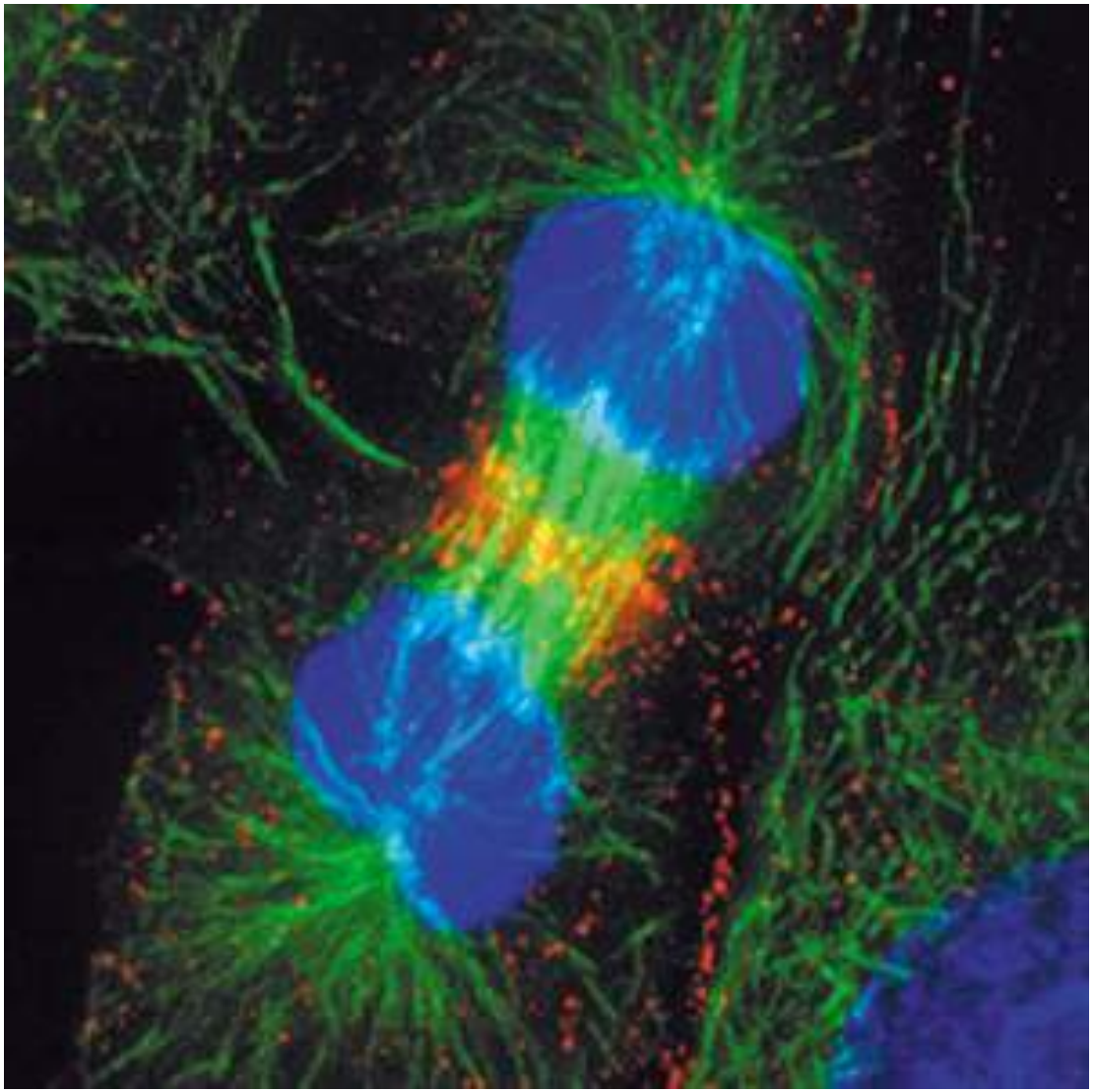
画像化・視覚化

染色イメージング



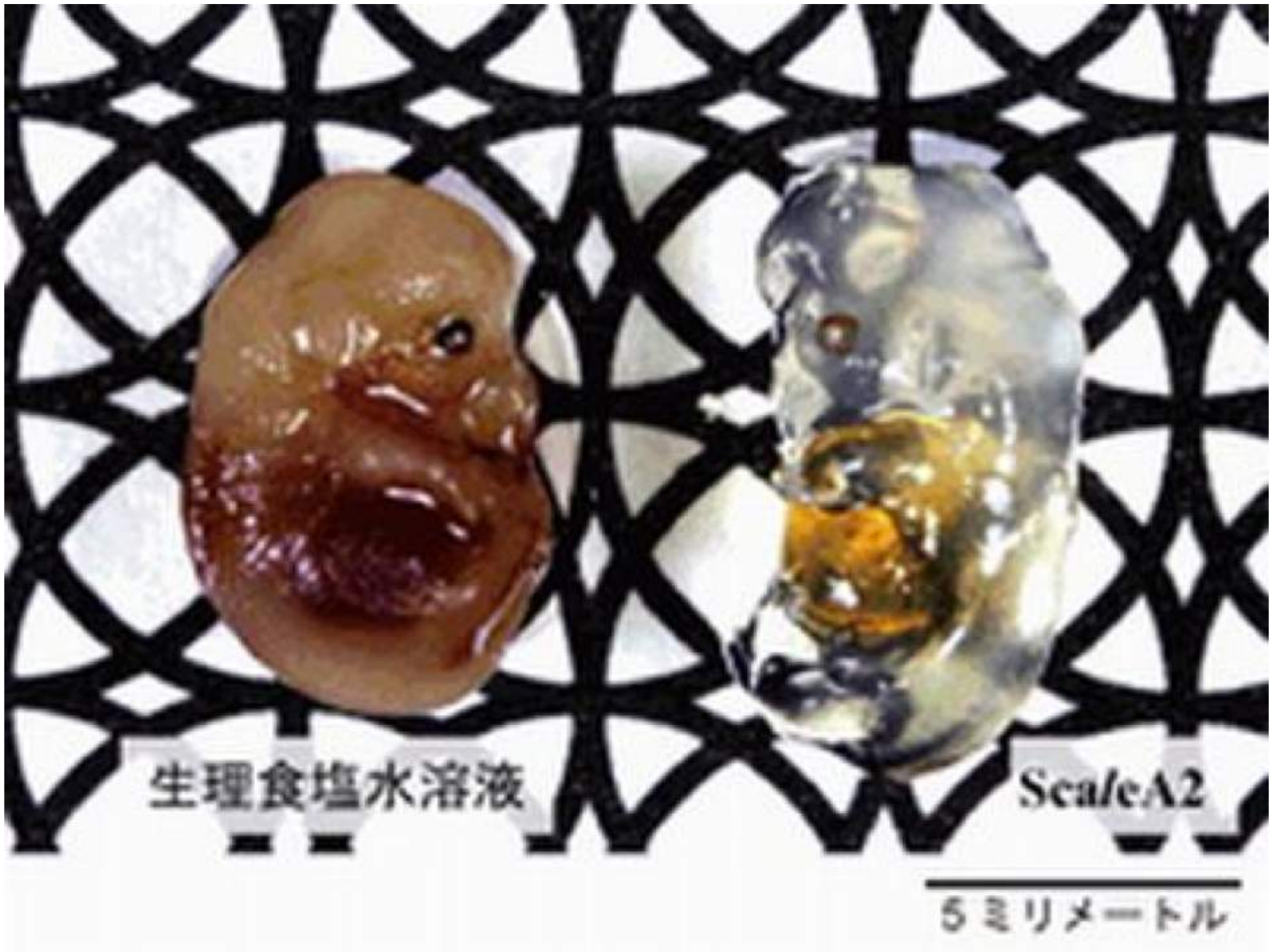
酢酸カーミンで染めた核

蛍光染色イメージング

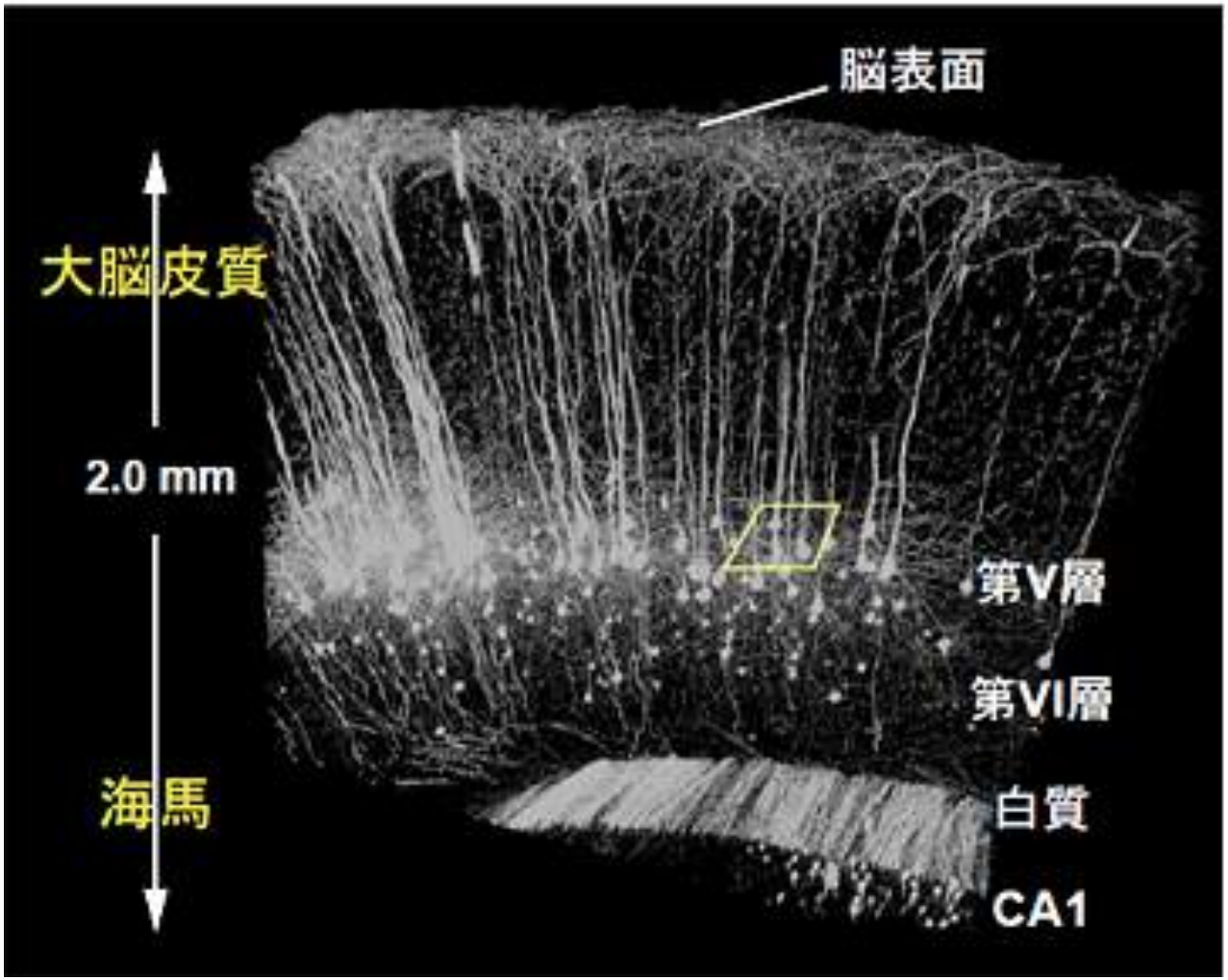


イメージングの先端技術（透明化技術）

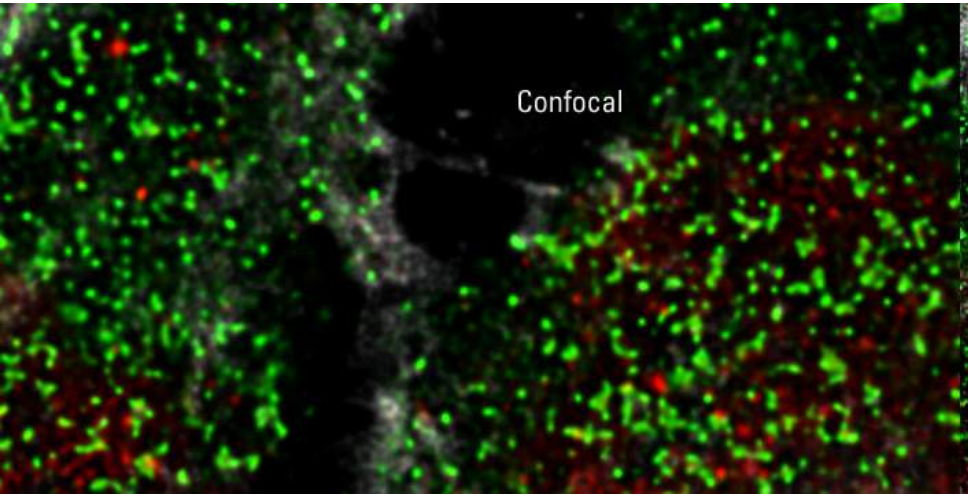
透明化試薬（スケール）

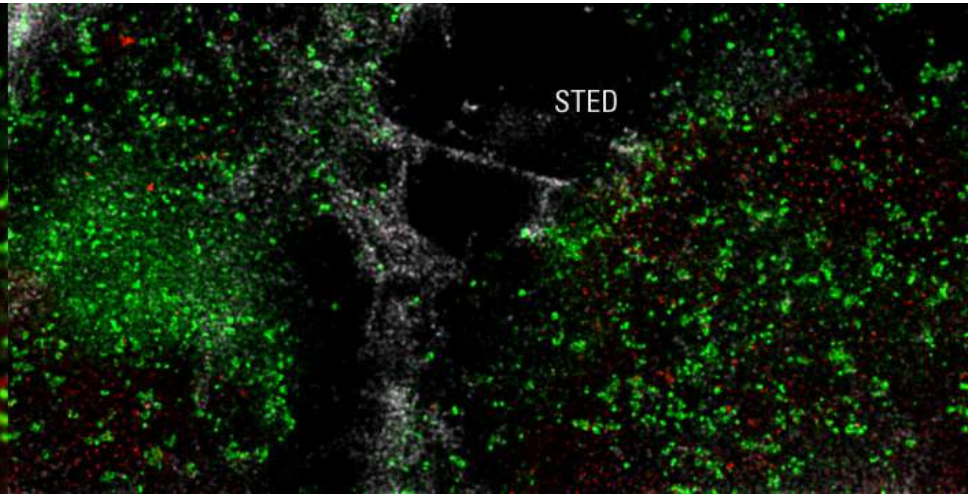


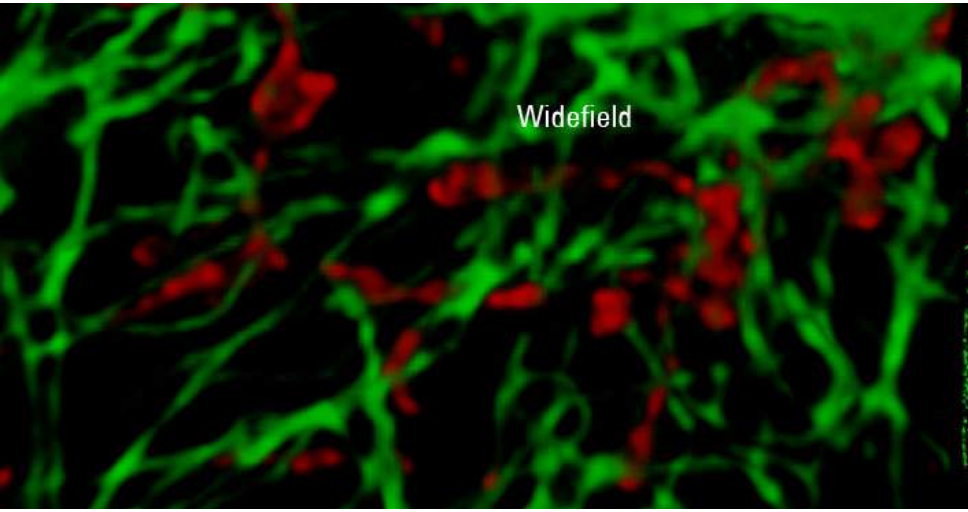
イメージングの先端技術（透明化技術）

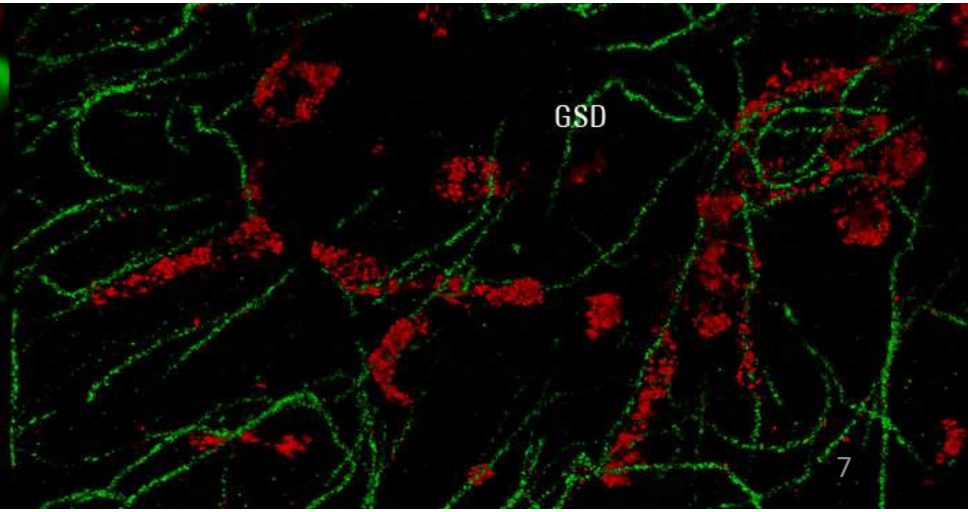


イメージングの先端技術
超解像顕微鏡：ノーベル化学賞2014









“ライブ”イメージング

・生きている細胞を使って可視化する

発光タンパク質

蛍光タンパク質

Extraction, Purification and Properties of Aequorin, a Bioluminescent Protein from the Luminous Hydromedusan, *Aequorea*[1]

OSAMU SHIMOMURA,[2] FRANK H. JOHNSON AND YO SAIGA
Department of Biology, Princeton University, Princeton, New Jersey, and the Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington

In experiments that have become classic in bioluminescence, Dubois (1885, 1887), first prepared from a luminous elaterid, *Pyrophorus*, and a luminous clam, *Pholas*, respectively, crude extracts containing a substrate, luciferin, and an enzyme, luciferase, which luminesced on mixing in aqueous solution containing dissolved oxygen. In the years that have followed, efforts have repeatedly been made to separate functionally similar components from numerous other, divers types of luminous organisms (Harvey, '52; '55). Although the majority of these efforts proved unsuccessful, about a dozen biologically specific, chemically different luciferin-luciferase systems have by now been obtained in varying degrees of purification (Johnson, Sie and Haneda, '61). The present investigation has resulted in the discovery of a new type of luminescent system, differing from those hitherto extracted in being comprised of a single organic component, with the properties of a protein. In aqueous solution either devoid of, or saturated with, oxygen, this protein gives a light-emitting reaction on addition of Ca^{++}. With the procedures employed, nearly 10,000 individual specimens of the hydromedusan, *Aequorea*, yielded about 5 mg of the highly purified, active substance which we have named "Aequorin" (Shimomura, Johnson and Saiga, '62).

While certain aspects of the phenomenon of luminescence in medusae have long been known, the biochemistry involved has remained very much of an enigma. As early as the first century, Pliny (cf. Harvey, '57) described the light of "Pulmo Marinus," evidently *Pelagia noctiluca*, and observed that luminous slime from the bell could be rubbed onto various surfaces making them glow as if on fire. Spallanzani (1794, 1798) noted that luminescence of this organism continued after death, and that a dark, almost liquefied specimen luminesced on addition of fresh water. At the turn of the nineteenth century, von Humboldt (1799–1804; cf. von Humboldt, 1853) and Macartney (1810) found that luminescence of medusae could be elicited by electrical stimuli, a phenomenon that has only recently been investigated from a modern viewpoint (Davenport and Nicol, '55; Nicol, '60). Macartney (1810) also found that no diminution in luminescence could be detected in a vacuum, as compared to aerobic conditions. The lack of a free oxygen requirement for luminescence was convincingly demonstrated by Harvey ('26; Harvey and Korr, '38), not only for medusae but also radiolarians and ctenophores. Luminescence under strictly anaerobic conditions, however, is an unusual feature among the various types of luminescent systems now known (Harvey, '52; '55).

Though unsuccessful in attempts to obtain a luciferin - luciferase reaction with extracts of *Aequorea*, Harvey ('21) found that the photogenic tissues dried over $CaCl_2$ would luminesce when moistened. Moreover, strips of the margin of the umbrella, where the photogenic organs are

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[1] Aided in part by National Science Foundation grant number G-12857, Office of Naval Research Contract Nonr 1353(00), Project NR 165-253, and the Eugene Higgins Fund allocated to Princeton University. Publication in whole or in part by or for any purpose of the United States Government is permitted.
[2] On leave from the Pharmacy Department, University of Nagasaki, Japan.

副産物として偶然に単離された緑色蛍光タンパク質（GFP）

Extraction, Purification and Properties of Aequorin, a Bioluminescent Protein from the Luminous Hydromedusan, *Aequorea*[1]

(Hirata, Shimomura an

Ultraviolet absorpt

Except for a slight bu
ultraviolet absorption sp
similar to that of simpl
peak at 280 mμ. After t
action the bulge at 310 r
a new absorption maxim
333 mμ.

Requirement of Ca^{++} for luminescence, and effects of other cations

The calcium requirement has been referred to above. Thirteen other cations in the form of salts of chloride, sulfate or acetate were tested for a possible activat-

by the data
When the concen-

[3] A protein giving solutions that look slightly greenish in sunlight though only yellowish under tungsten lights, and exhibiting a very bright, greenish fluorescence in the ultraviolet of a Mineralite, has also been isolated from squeezates. No indications of a luminescent reaction of this sustance could be detected. Studies of the emission spectra of both this protein and aequorin are in progress.

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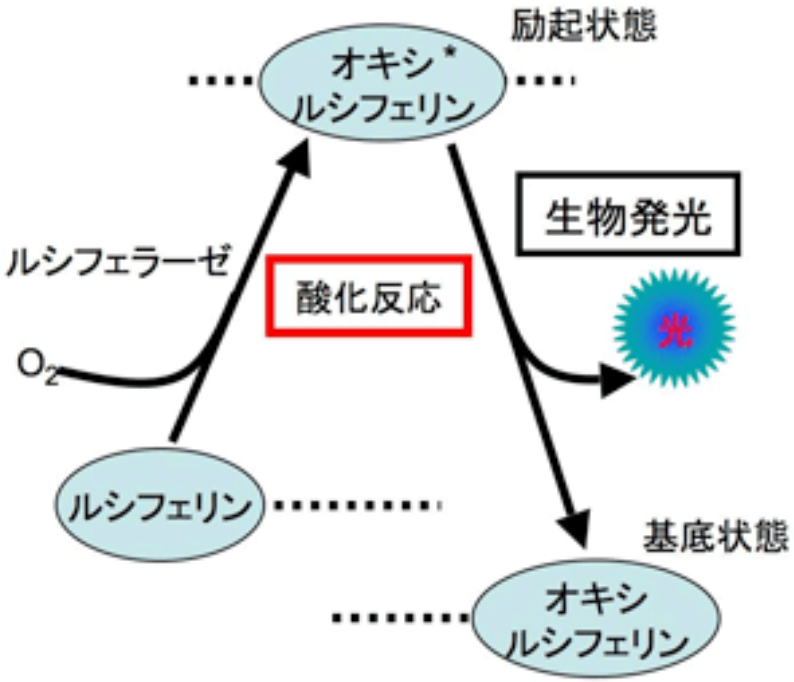
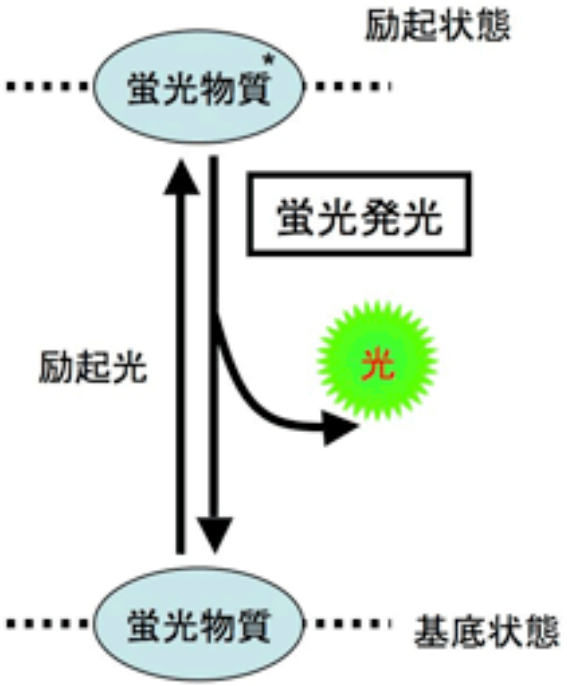
生物発光と蛍光の違い

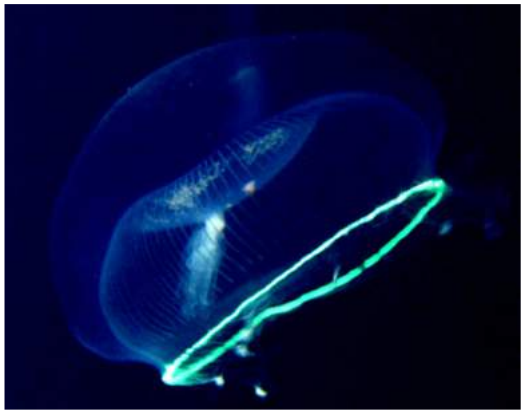

励起状態
オキシ*
ルシフェリン
生物発光
ルシフェラーゼ
酸化反応
光
O_2
ルシフェリン
基底状態
オキシ
ルシフェリン


励起状態
蛍光物質*
蛍光発光
励起光
光
蛍光物質
基底状態

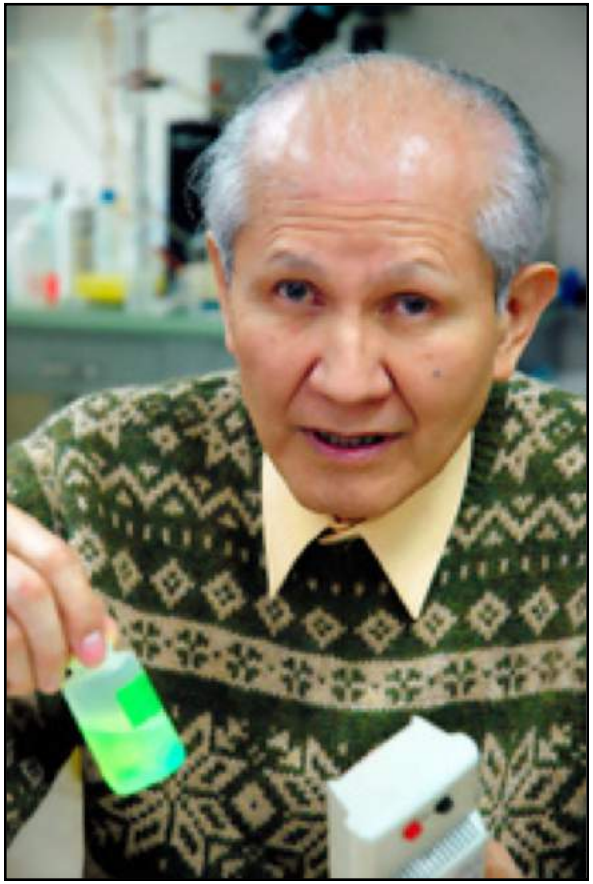
蛍光タンパク質

Green Fluorescent Protein (GFP)

オワンクラゲ



下村脩　博士



2008年　ノーベル化学賞

Osamu Shimomura
下村　脩

1962年
オワンクラゲから発見

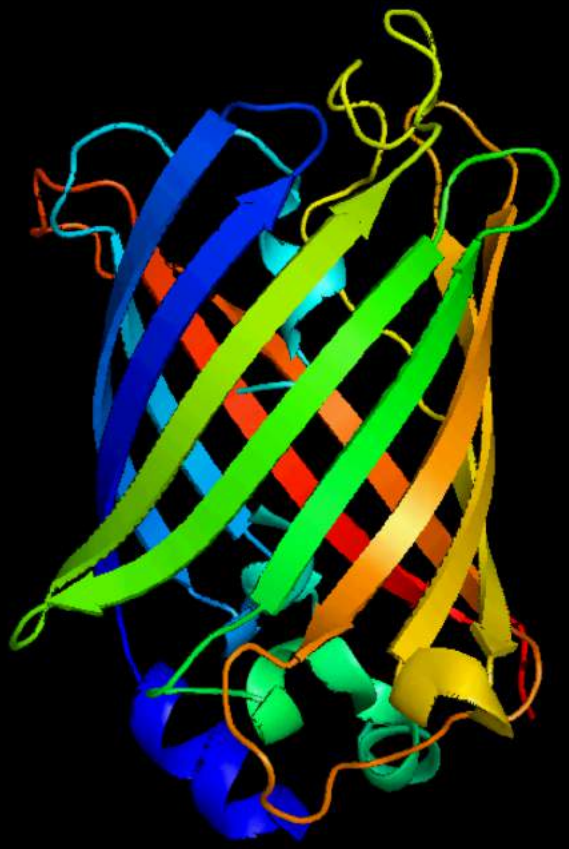
Martin Chalfie

1994年
異種生物で発現

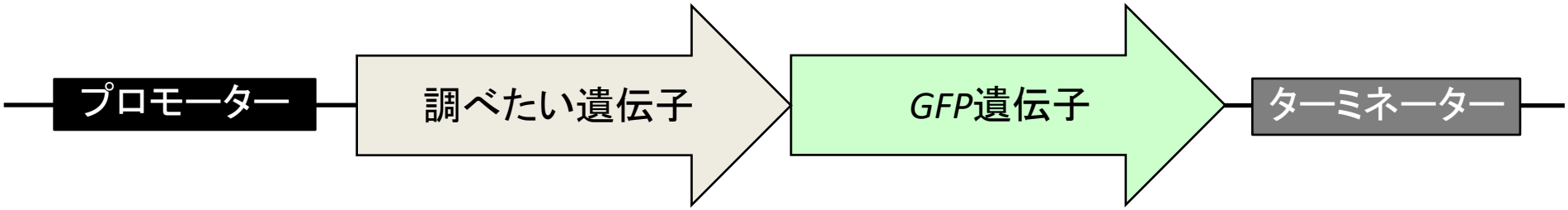
Roger Tsien

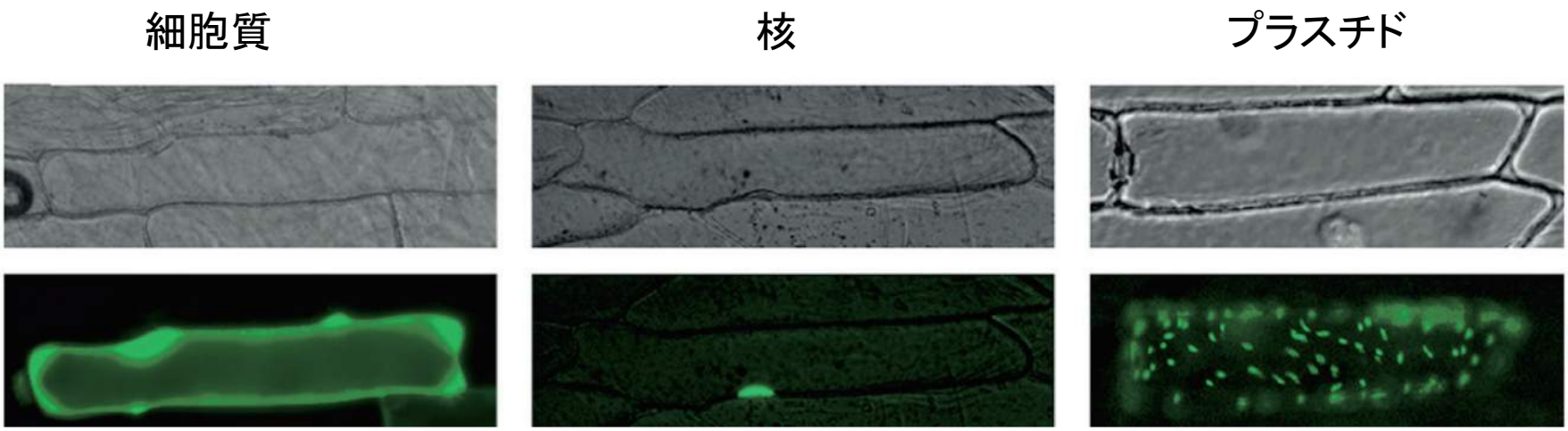
1995年～現在
蛍光タンパク質の改良

蛍光タンパク質

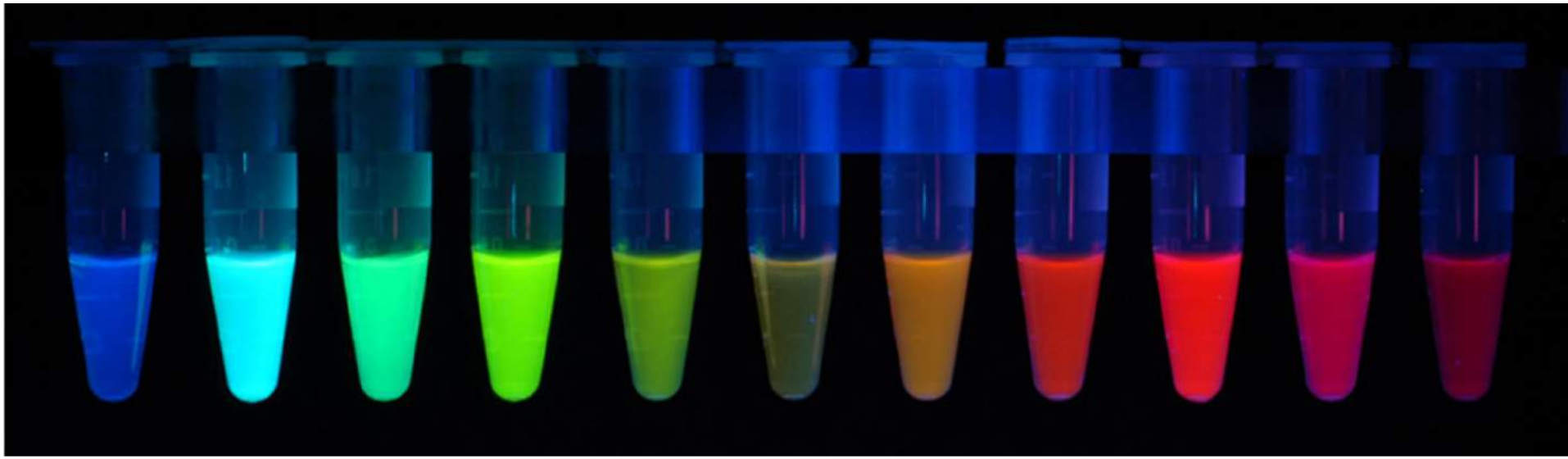


蛍光タンパク質の設計図DNAを使って細胞内のタンパク質の存在場所を視る



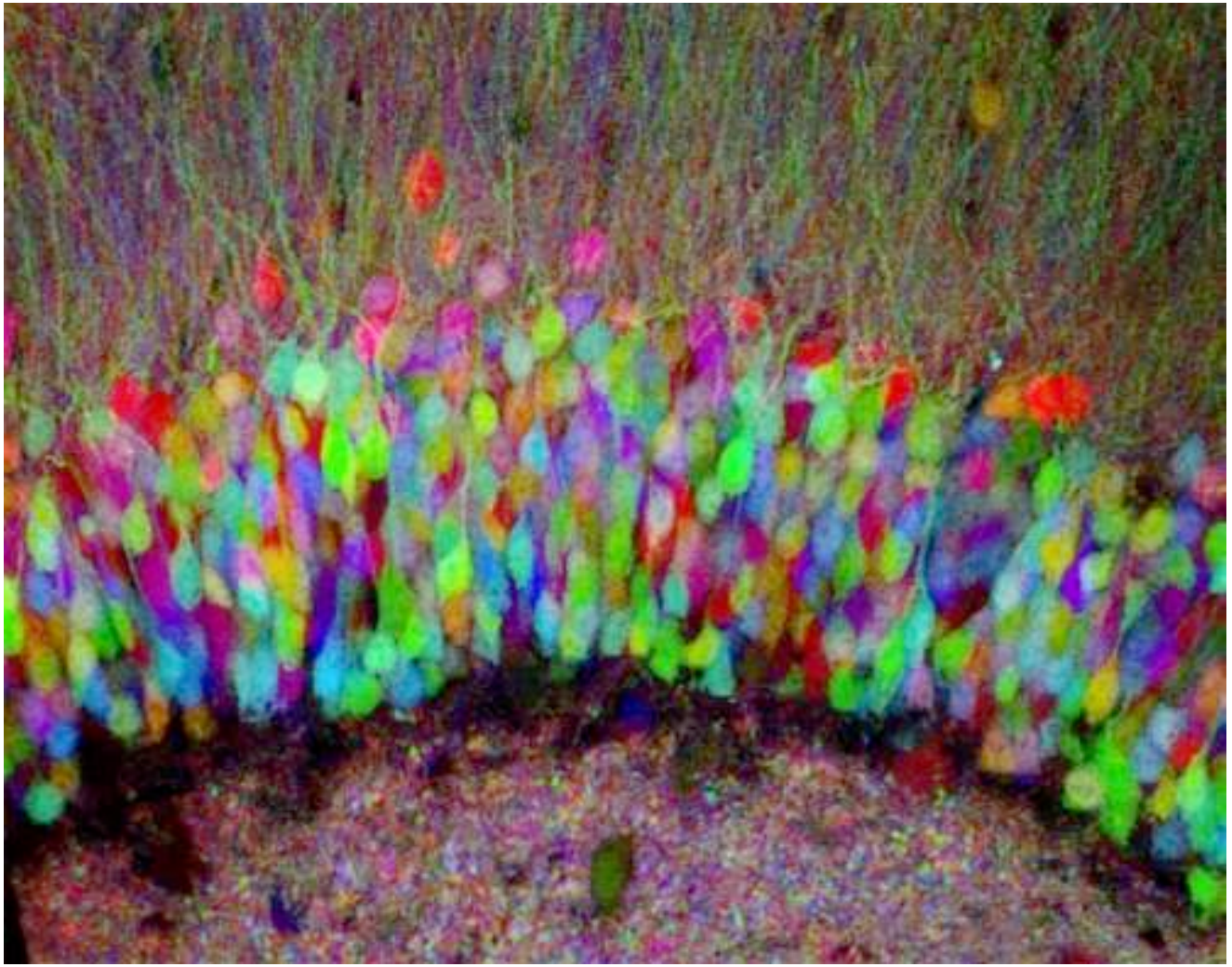


様々な色、特徴を持つ蛍光タンパク質が開発されている



Tsien Lab ホームページより転載

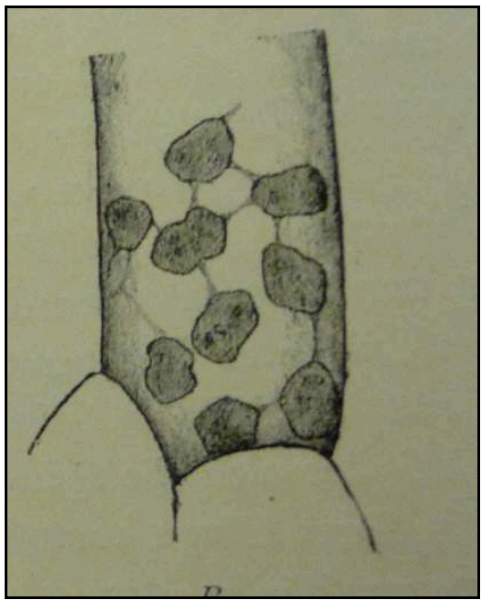
神経の可視化：Brainbow

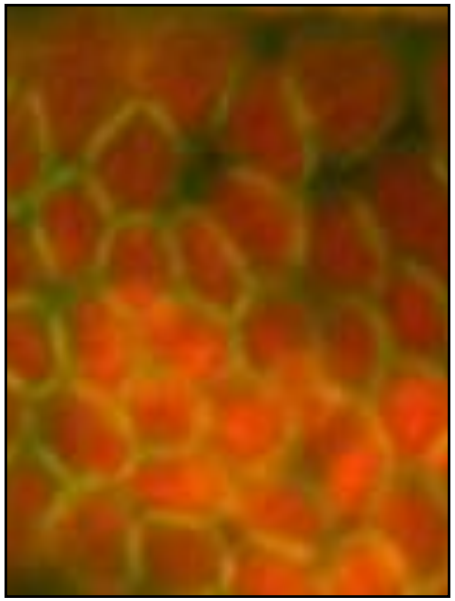


Livet et al. (2007) Nature

過去・現在・未来のバイオイメージング

約20℃





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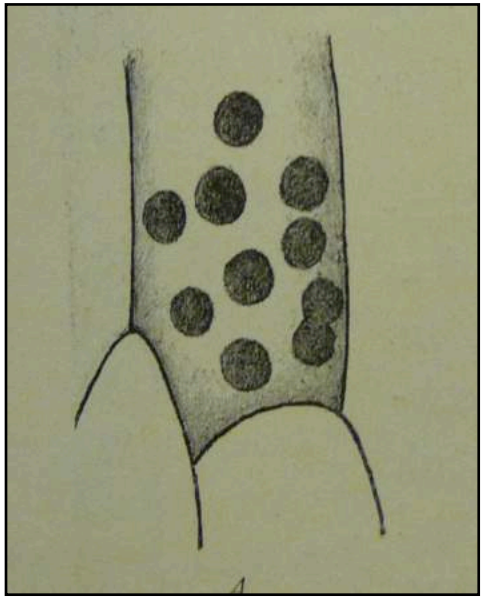
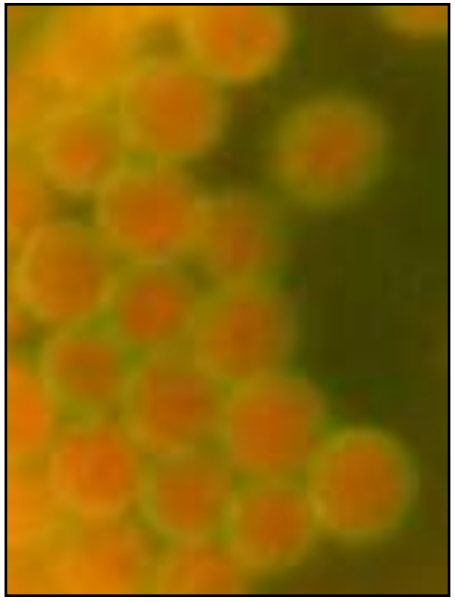
約0℃





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