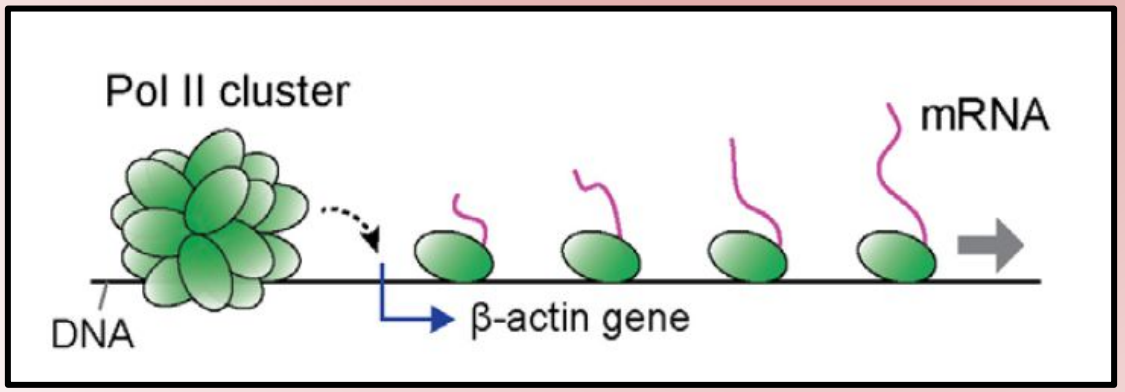
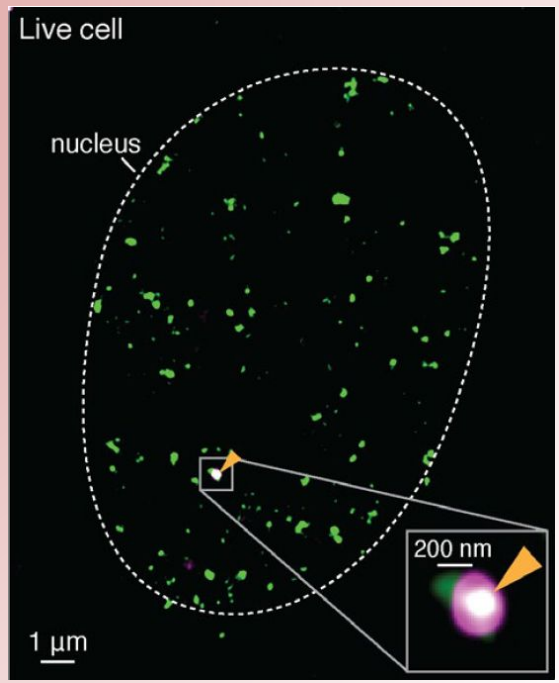
Ibrahim Cissé is a **biophysicist** who uses **single molecule** and **super-resolution microscopy** techniques to visualize **gene expression** and **dynamic processes** in living cells.





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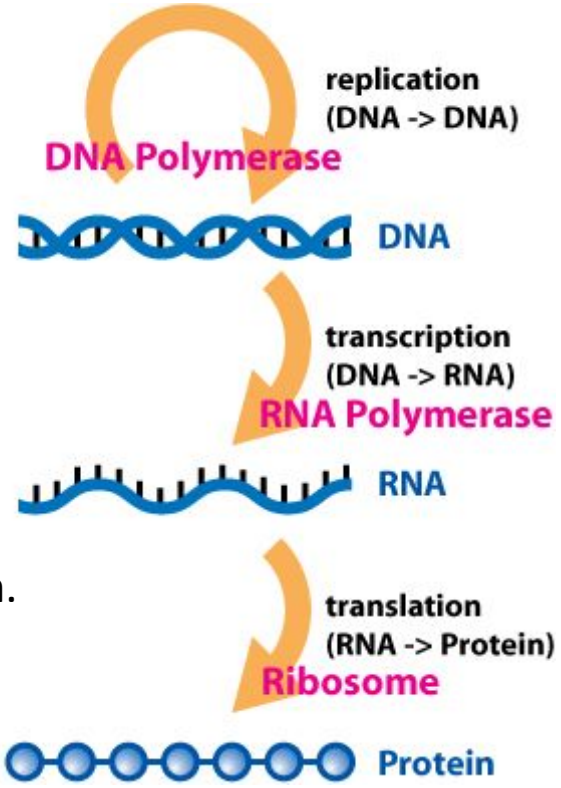
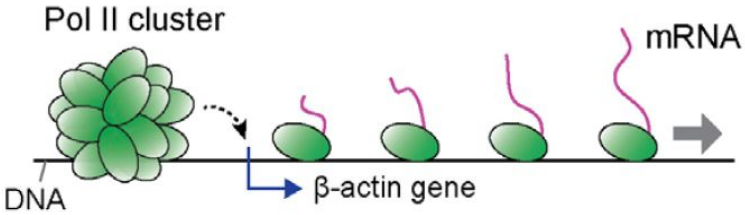
The **central dogma of molecular biology** describes the process by which genetic information flows within the cell of a living organism.

There are two key stages of **gene expression**:

1. **Transcription**: DNA is copied to RNA.
2. **Translation**: proteins are synthesized from information encoded in RNA.



RNA polymerase II (**Pol II**) is an **enzyme** which reads the information in DNA and converts it to RNA during transcription.



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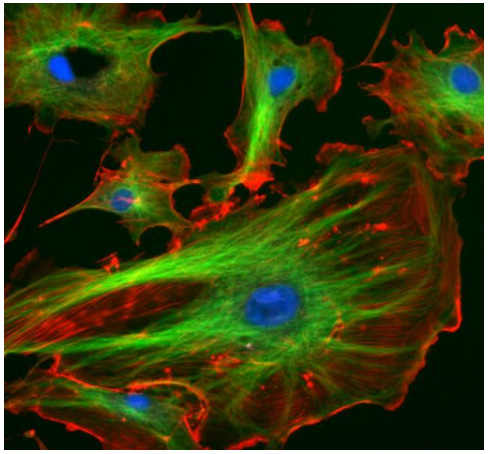
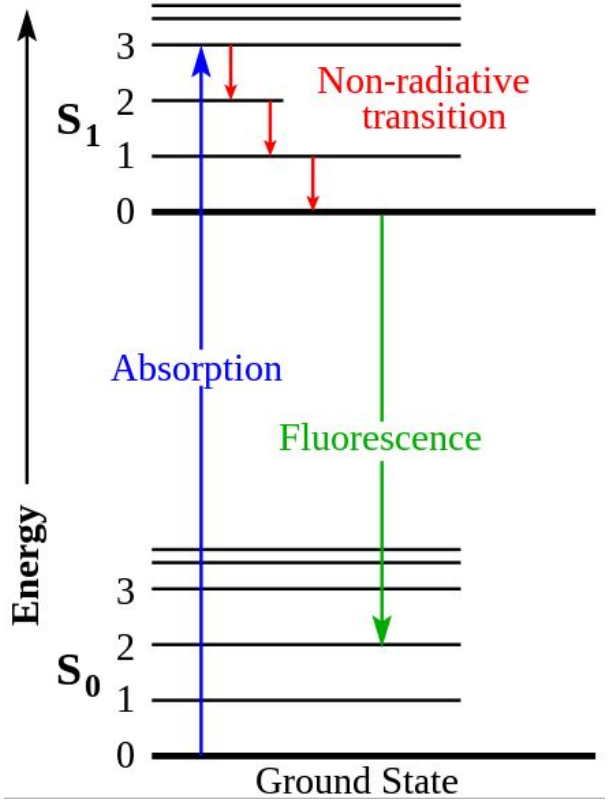


Fluorescence microscopy is a type of optical microscopy that uses fluorescence to generate images of organic or inorganic substances.

Fluorescence is the **emission** of light by a substance after **absorbing** light or other electromagnetic radiation.



The **diffraction limit** of light constrains the size of the features that can be imaged by a microscope to a **few hundred nanometers** when using visible light.

Sub-diffraction resolution is needed to study the **biomolecules** (which are a **few nanometers** in scale) that make up the molecular machines in living cells.

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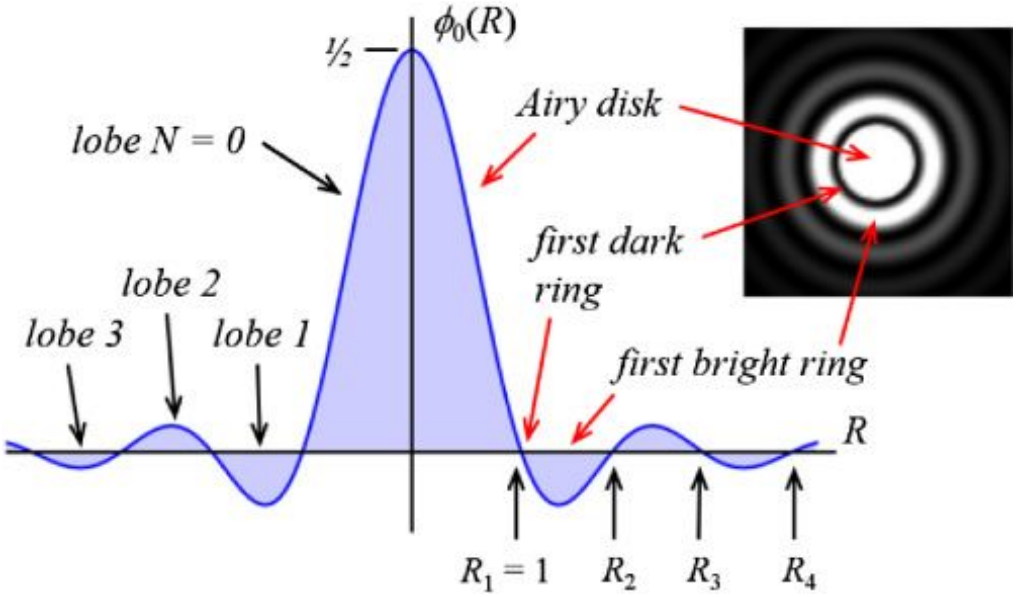
The size of the smallest feature imaged by a microscope with a circular aperture is limited to the size of the **Airy disk**.

The **minimum resolvable distance** *d* for light with a wavelength *λ* seen by a microscope with numerical aperture *NA* is given by the **Abbe diffraction limit**:

$$d = \frac{\lambda}{2NA}$$



A point source is usually localized by fitting the emission profile to a **Gaussian**.

If *n* photons are captured, the **localization accuracy** is: $$\Delta d = \frac{\lambda}{2NA\sqrt{n}}$$

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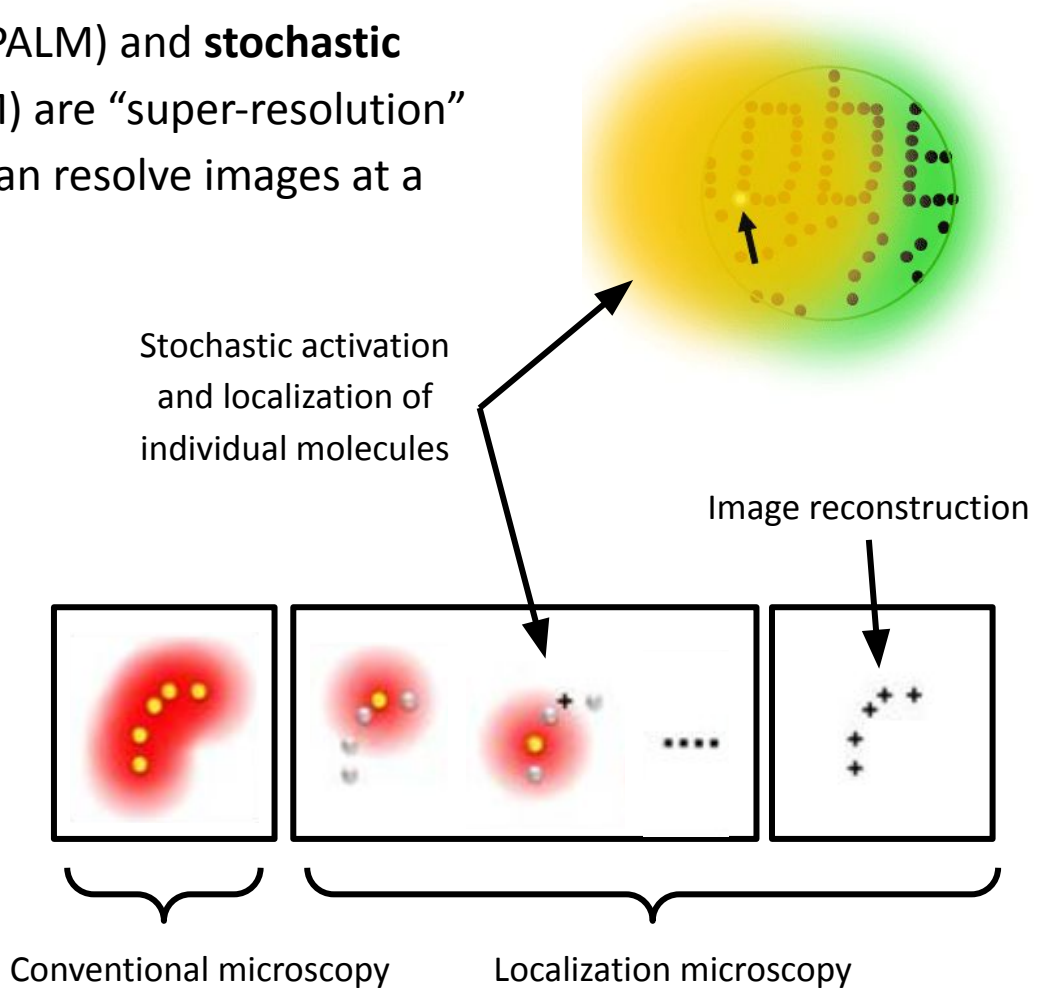
Photo-activated localization microscopy (PALM) and **stochastic optical reconstruction microscopy** (STORM) are "super-resolution" fluorescence microscopy techniques that can resolve images at a higher resolution than the diffraction limit.

PALM/STORM take many images of a specimen with different molecules **stochastically switching** from (dark) **off-states** to (fluorescent) **on-states**.

The molecules' positions are **localized** to create **super-resolution** images. This takes time, restricting observations to **static biological samples**.



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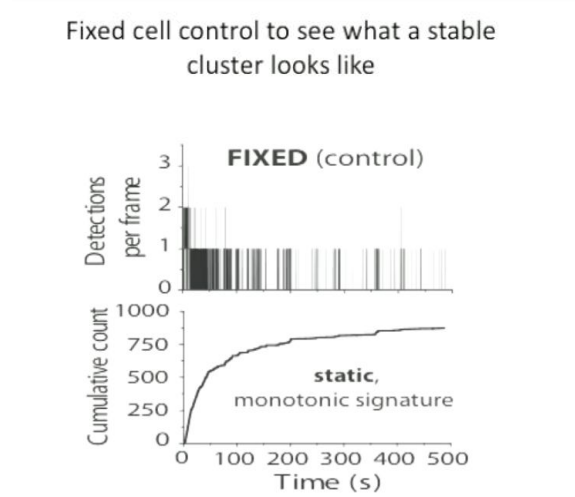
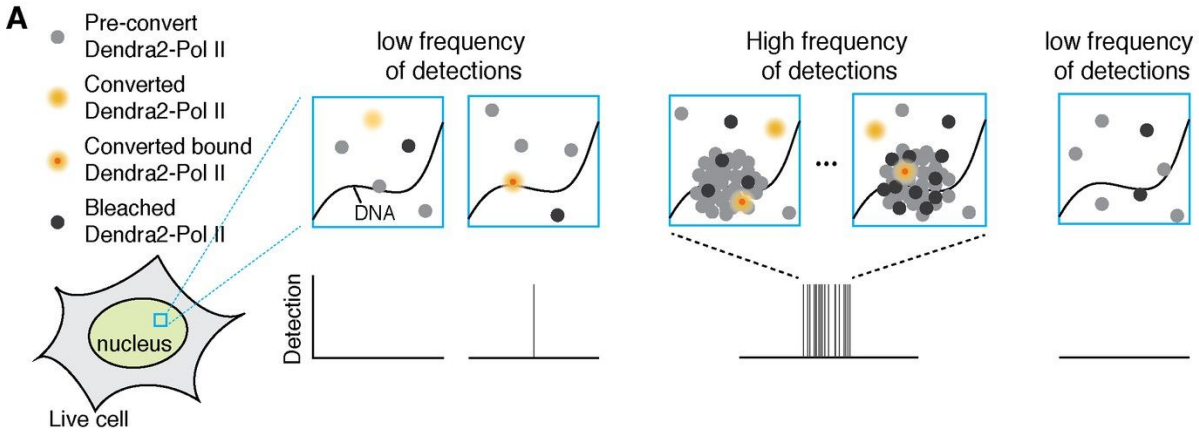
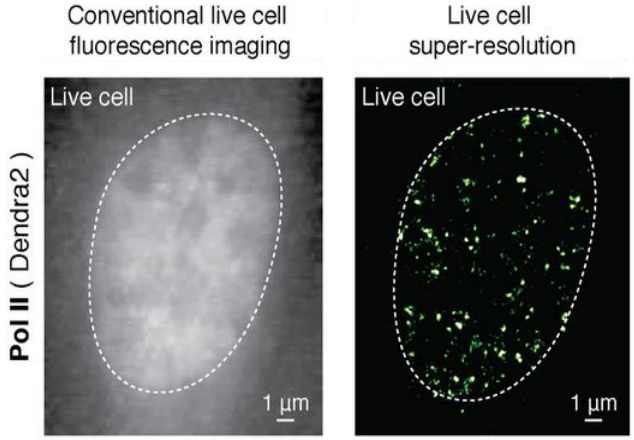
Cissé's group developed **time-correlated photo-activation microscopy** (tcPALM) to do **super-resolution** imaging of **dynamic** biological samples.

They studied the function of **Pol II clusters** in living cells and made a new discovery of a mechanism for **gene regulation**.







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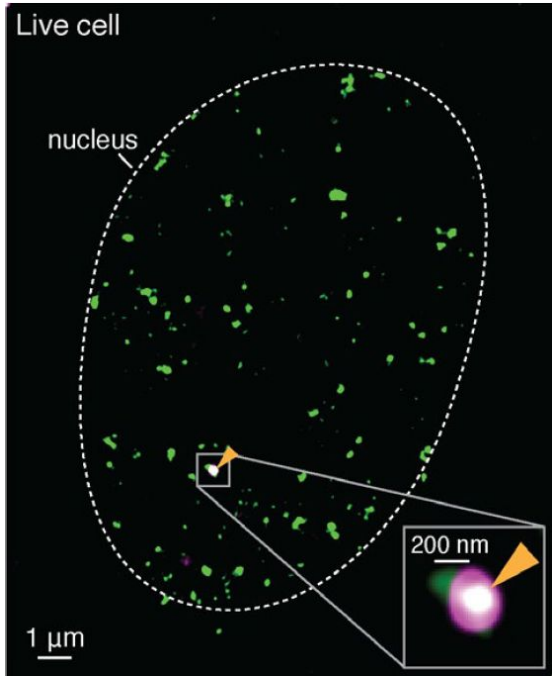
Cissé's group used **tcPALM** to measure **Pol II dynamics** in living cells and discovered that Pol II formed clusters of 100 molecules that would disassemble after a **few seconds**: orders of magnitude shorter than the time it takes to Pol II to copy a gene.



Using **dual color super-resolution imaging**, they discovered that the Pol II cluster lifetimes **correlated linearly** with the number of RNA copies produced.