

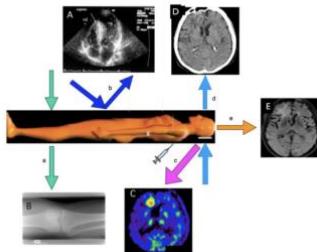


TP

Biomedical Imaging

## Question 1:

Complétez le figure en précisant les différentes modalités d'imagerie médicale (A,..., E) ainsi que le type de signal recueilli (a,..., e). Aidez-vous des flèches



### Identification of Each Modality:

Image (Label)	Imaging Modality	Physical Principle / Signal Type	Explanation
A	Échographie (Ultrasound Imaging)	Propagation et réflexion d'ondes ultrasonores	Ultrasound uses high-frequency sound waves (1–20 MHz) that are reflected by tissue interfaces. The reflected echoes are converted into an image in real time. It's a non-invasive, low-cost, and real-time technique widely used for cardiac and obstetric imaging.
B	Radiographie (X-ray Imaging)	Atténuation des rayons X	X-rays pass through the body and are absorbed differently by tissues depending on their density (bones, muscles, air). The resulting image shows contrast based on this attenuation, useful for bones and lungs.
C	TEP (Tomographie par Émission de Positons / PET)	Émission de photons $\gamma$ par un traceur radioactif	PET imaging detects gamma rays emitted by a radioactive tracer injected into the patient. It gives information about metabolic and functional activity rather than anatomy.
D	Scanner (Tomodensitométrie / CT)	Mesure de l'atténuation des rayons X dans plusieurs directions	CT reconstructs 3D images from multiple X-ray projections. It provides detailed anatomical information with higher resolution than standard radiography.
E	IRM (Imagerie par Résonance Magnétique)	Signal radiofréquence émis par les protons excités dans un champ magnétique	MRI measures signals from atomic nuclei (mainly hydrogen) placed in a strong magnetic field. It provides excellent soft tissue contrast and can capture both anatomical and functional data.

### Summary Table:

Modality	Label	Signal
Radiographie	B	X-rays
Échographie	A	Ultrasound waves (mechanical acoustic waves)
TEP (PET)	C	Gamma photons (emitted radiation)
Scanner (CT)	D	X-rays (ionizing electromagnetic radiation)
IRM	E	Radiofrequency electromagnetic signal

## Question 2:

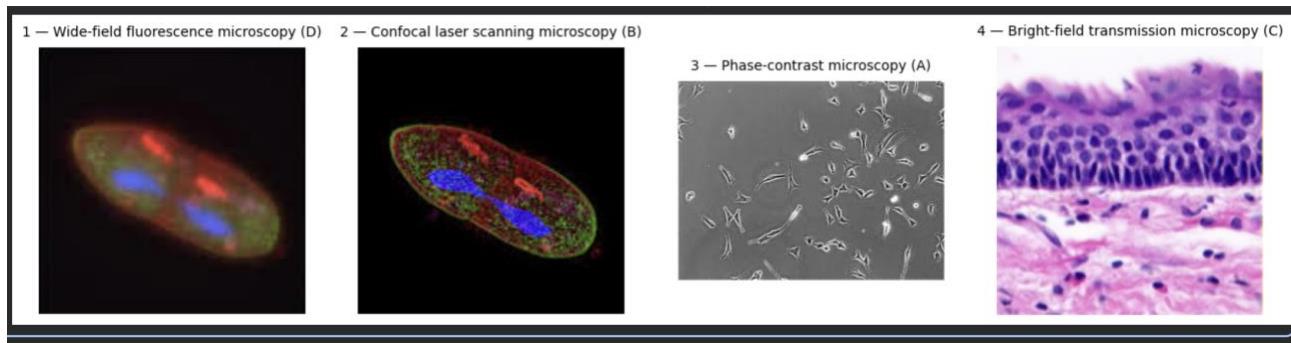
Faites un schéma similaire pour la microscopie photonique. A) Microscopie à contraste de phase. B) microscopie confocale à balayage. C) Microscopie en transmission, champs clair. D) Microscopie à fluorescence, à champs large.

### Introduction

The figure below summarizes four major techniques in light (photon) microscopy. Each technique uses visible light or fluorescence to form images but relies on different optical principles to generate contrast.

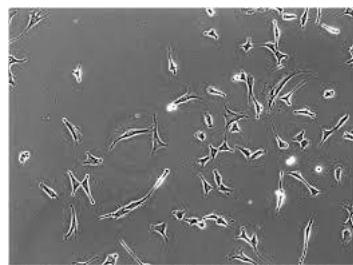
The four methods are:

- A) Phase-contrast microscopy,
- B) Confocal laser scanning microscopy,
- C) Bright-field transmission microscopy, and
- D) Wide-field fluorescence microscopy.



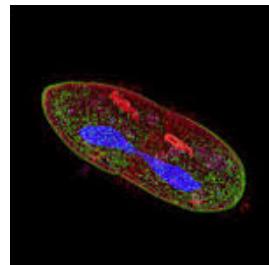
#### A) Microscopie à contraste de phase (Phase-contrast microscopy)

- **Type of light/signal:** Transmitted visible light.
- **Main components:** Light source, condenser with phase annulus, specimen, objective lens with phase plate, detector/eyepiece.
- **Principle:** This technique converts tiny phase shifts of light, caused by variations in the refractive index within the specimen, into intensity differences visible to the human eye. It allows observation of unstained, transparent, living cells with enhanced internal detail.



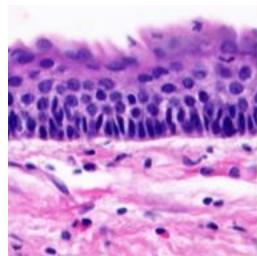
#### B) Microscopie confocale à balayage (Confocal laser scanning microscopy)

- **Type of light/signal:** Laser excitation and fluorescence emission.
- **Main components:** Laser source, scanning mirrors, objective lens, specimen, pinhole, photodetector.
- **Principle:** A focused laser beam scans the sample point-by-point. The pinhole blocks out-of-focus light, so only fluorescence from the focal plane is detected. This produces optical sections with high resolution and allows 3D reconstruction of fluorescent samples.



#### C) Microscopie en transmission, champ clair (Bright-field transmission microscopy)

- **Type of light/signal:** Transmitted white light.
- **Main components:** White light source, condenser, stained specimen, objective lens, eyepiece/camera.
- **Principle:** The image is formed by light transmitted through the sample. Contrast results from differences in absorption and scattering of light by stained regions. This is the standard technique used in histology to observe fixed, colored tissue sections.



#### D) Microscopie à fluorescence, champ large (Wide-field fluorescence microscopy)

- **Type of light/signal:** Excitation and emission of fluorescence.
- **Main components:** Excitation light source (lamp or LED), excitation filter, dichroic mirror, objective, fluorescent specimen, emission filter, detector/camera.
- **Principle:** The entire field is illuminated simultaneously to excite fluorophores in the sample. The emitted fluorescence (longer wavelength) is collected by the objective to form an image. This allows visualization of specific molecules or proteins using fluorescent labeling, although out-of-focus light can reduce contrast.



### Conclusion

Each light microscopy technique provides distinct advantages:

- **Phase-contrast:** visualizes live, unstained cells.
- **Bright-field:** shows stained tissue structure.

- **Wide-field fluorescence:** highlights labeled molecules.
- **Confocal:** offers sharp, 3D optical sections of fluorescent samples.

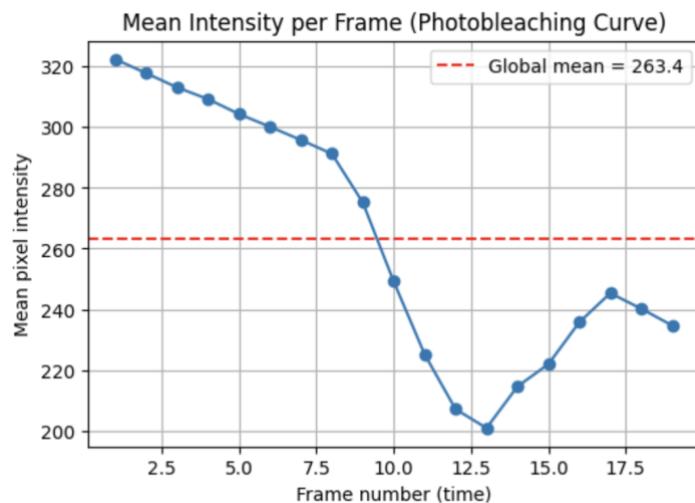
Together, these methods form the foundation of biological optical imaging and are essential for quantitative image analysis in microscopy.

### Question 3:

Calculer l'intensité moyenne de la séquence *cell2D\_timelapse.tif*. Quel phénomène physique pouvez-vous observer?

The mean fluorescence intensity of the sequence *cell2D\_timelapse.tif* was calculated for each of the 19 frames. The resulting curve (Figure) shows a continuous decrease in mean intensity from approximately 322 in frame 0 to 200. around frame 12, followed by a slight recovery toward 240 by the final frame. The global mean intensity across all frames is 263.

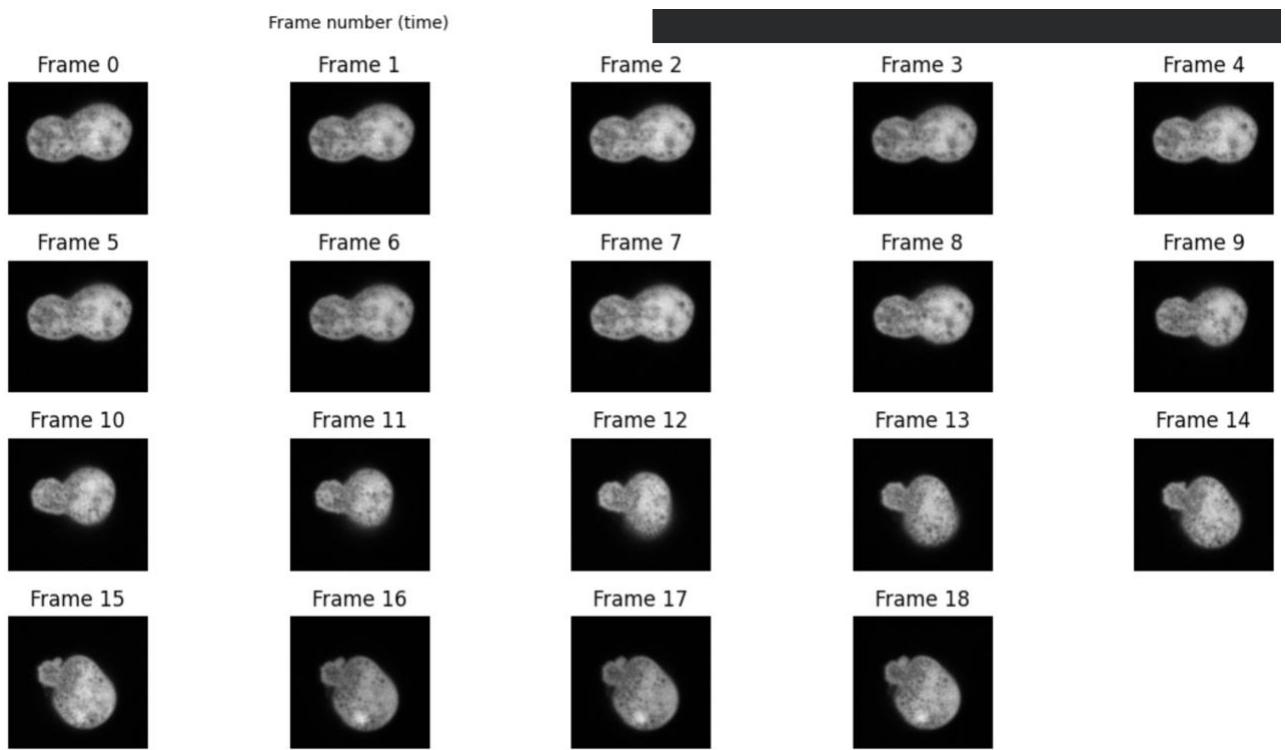
Photobleaching Curve:



This progressive loss of signal corresponds to a photobleaching phenomenon, a physical process in which fluorophores irreversibly lose their ability to emit light after repeated excitation. The nearly exponential decay observed in the first half of the timelapse is characteristic of bleaching due to prolonged exposure to the excitation light.

Toward the end of the sequence, a small increase in mean intensity is observed, which may result from cellular shape changes or redistribution of fluorescent molecules during what appears to be the onset of cell division. The morphological evolution visible in the frames supports this interpretation, showing the cell elongating and constricting at the center.

*Below are the frames observed*



*Mean Intensities across all frames:*

```
Frame 0: mean intensity = 322.41
Frame 1: mean intensity = 317.80
Frame 2: mean intensity = 313.11
Frame 3: mean intensity = 309.25
Frame 4: mean intensity = 304.34
Frame 5: mean intensity = 300.14
Frame 6: mean intensity = 295.80
Frame 7: mean intensity = 291.26
Frame 8: mean intensity = 275.20
Frame 9: mean intensity = 249.13
Frame 10: mean intensity = 225.13
Frame 11: mean intensity = 207.05
Frame 12: mean intensity = 200.84
Frame 13: mean intensity = 214.55
Frame 14: mean intensity = 221.91
Frame 15: mean intensity = 235.73
Frame 16: mean intensity = 245.30
Frame 17: mean intensity = 240.19
Frame 18: mean intensity = 234.65

Global mean intensity (all frames): 263.36
```

## Conclusion

The main physical phenomenon observed is photobleaching of the fluorescent signal over time, accompanied by visible morphological changes of the cell that may correspond to a division process.