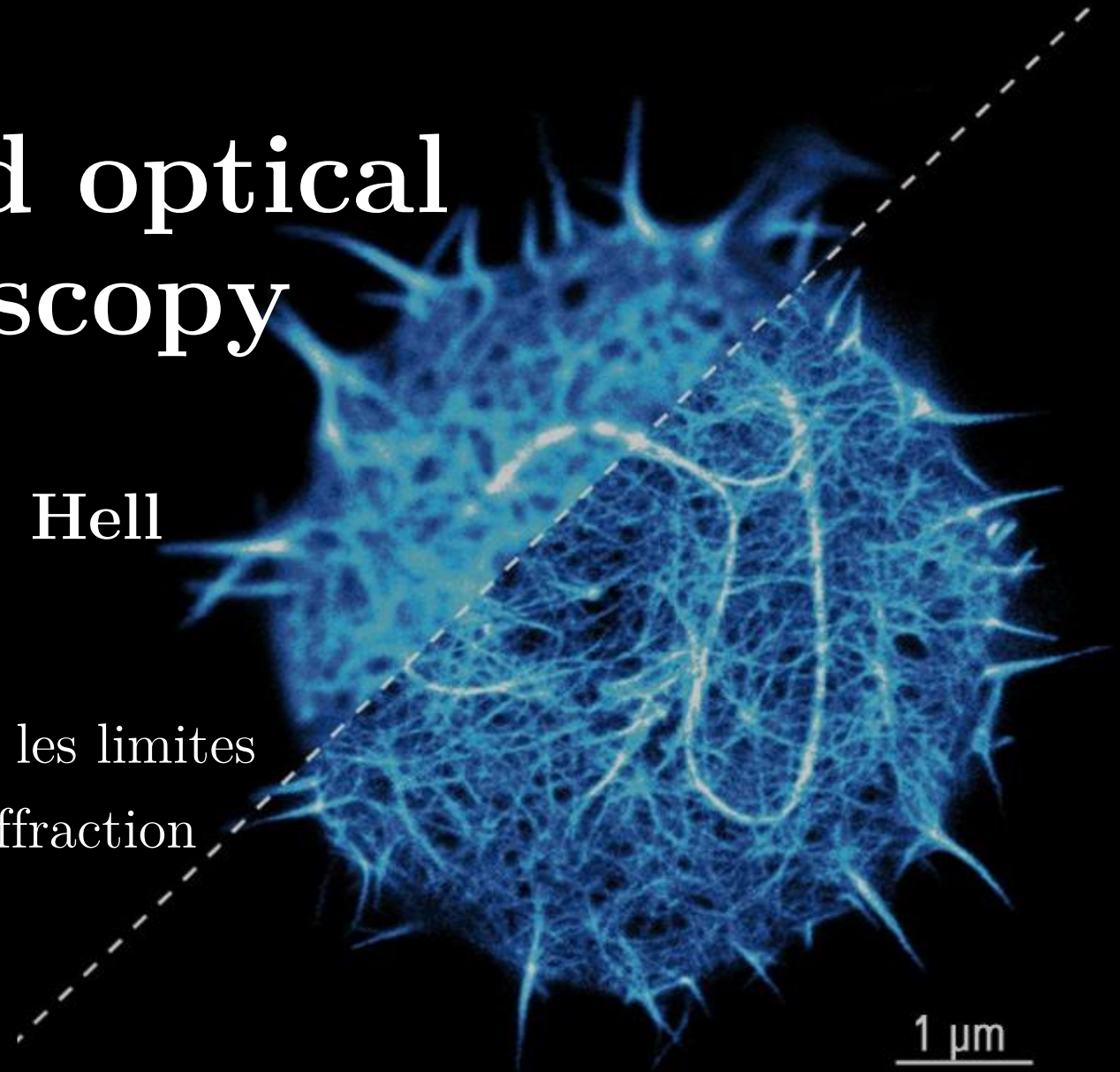


# Far-field optical nanoscopy

S.W. Hell

Repousser les limites  
de la diffraction

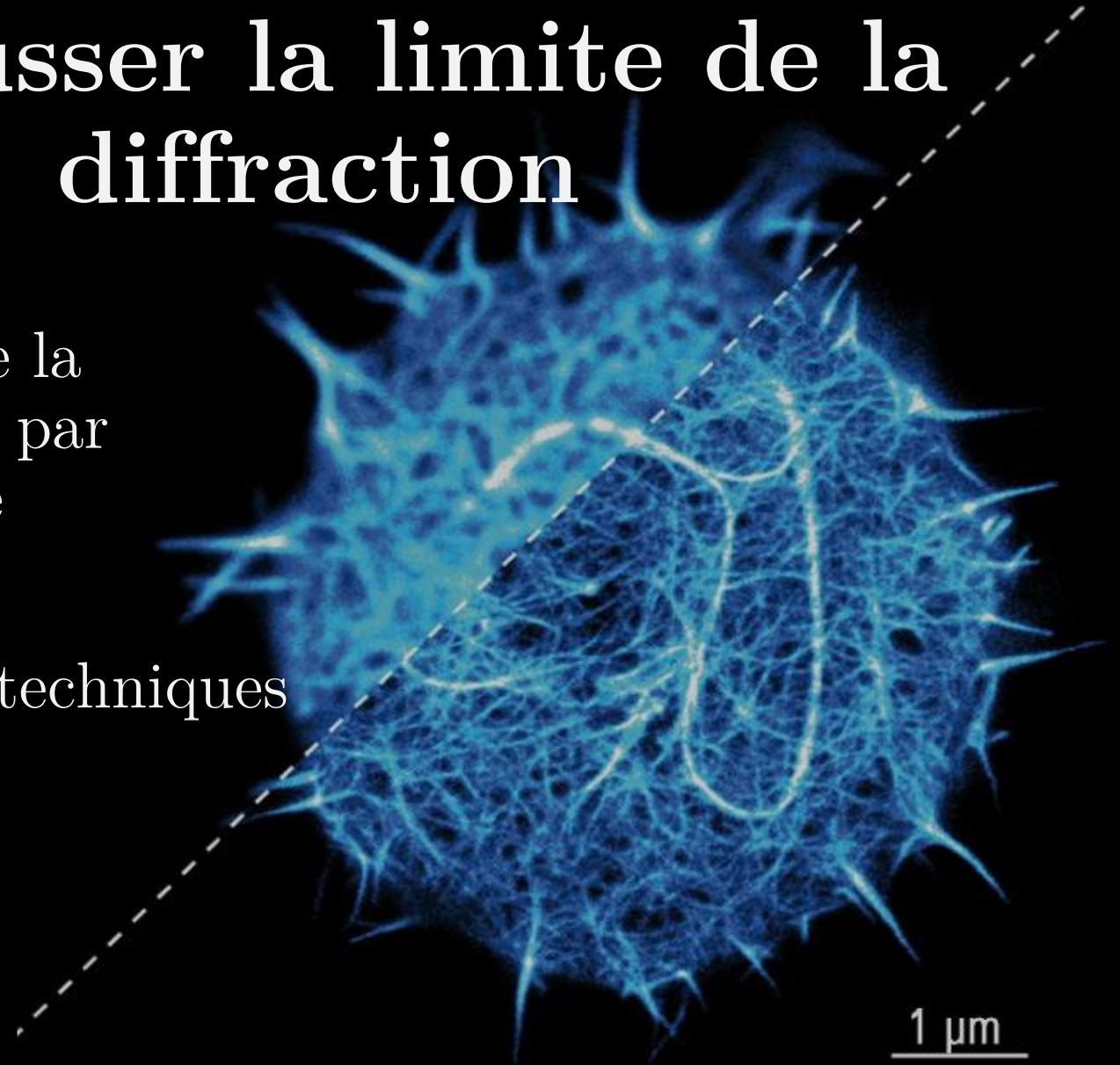


# Repousser la limite de la diffraction

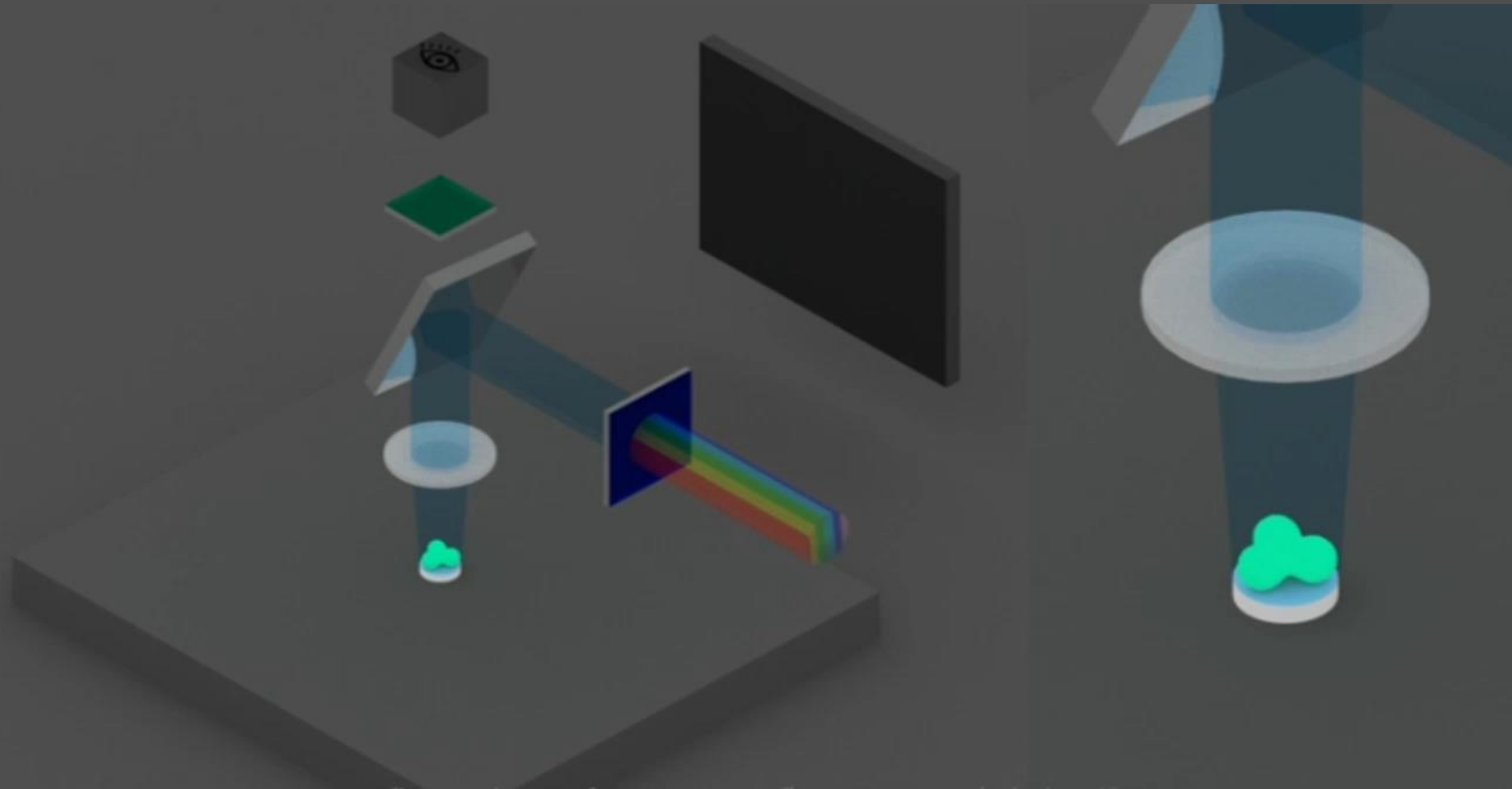
I. Contexte de la  
microscopie par  
fluorescence

II. Différentes techniques  
innovantes

1. STED
2. GSD
3. PALM

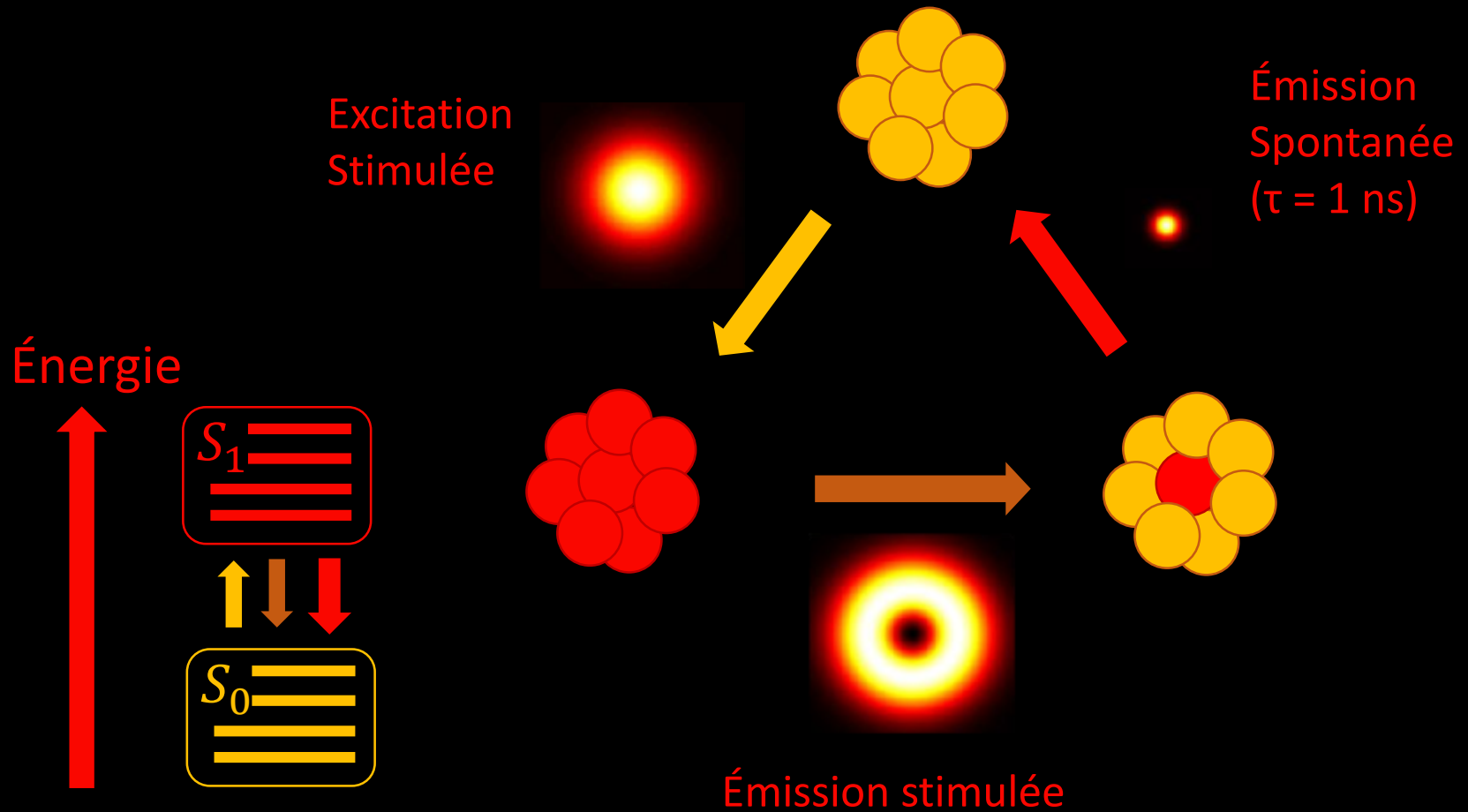


# Contexte de la microscopie par fluorescence :



... ces fluorophores émettent par fluorescence de la lumière avec une autre longueur d'onde (ici dans le vert).

# Innovations de la technique STED



# Innovations de la technique STED

Incertitude sur  
la position

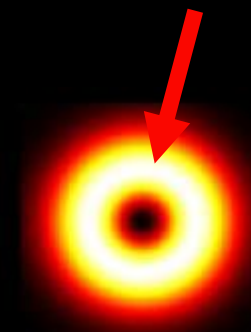
$$\Delta r \propto \frac{\lambda}{\sqrt{1 + \frac{I_{max}}{I_S}}}$$

Longueur  
D'onde

Intensité  
maximale

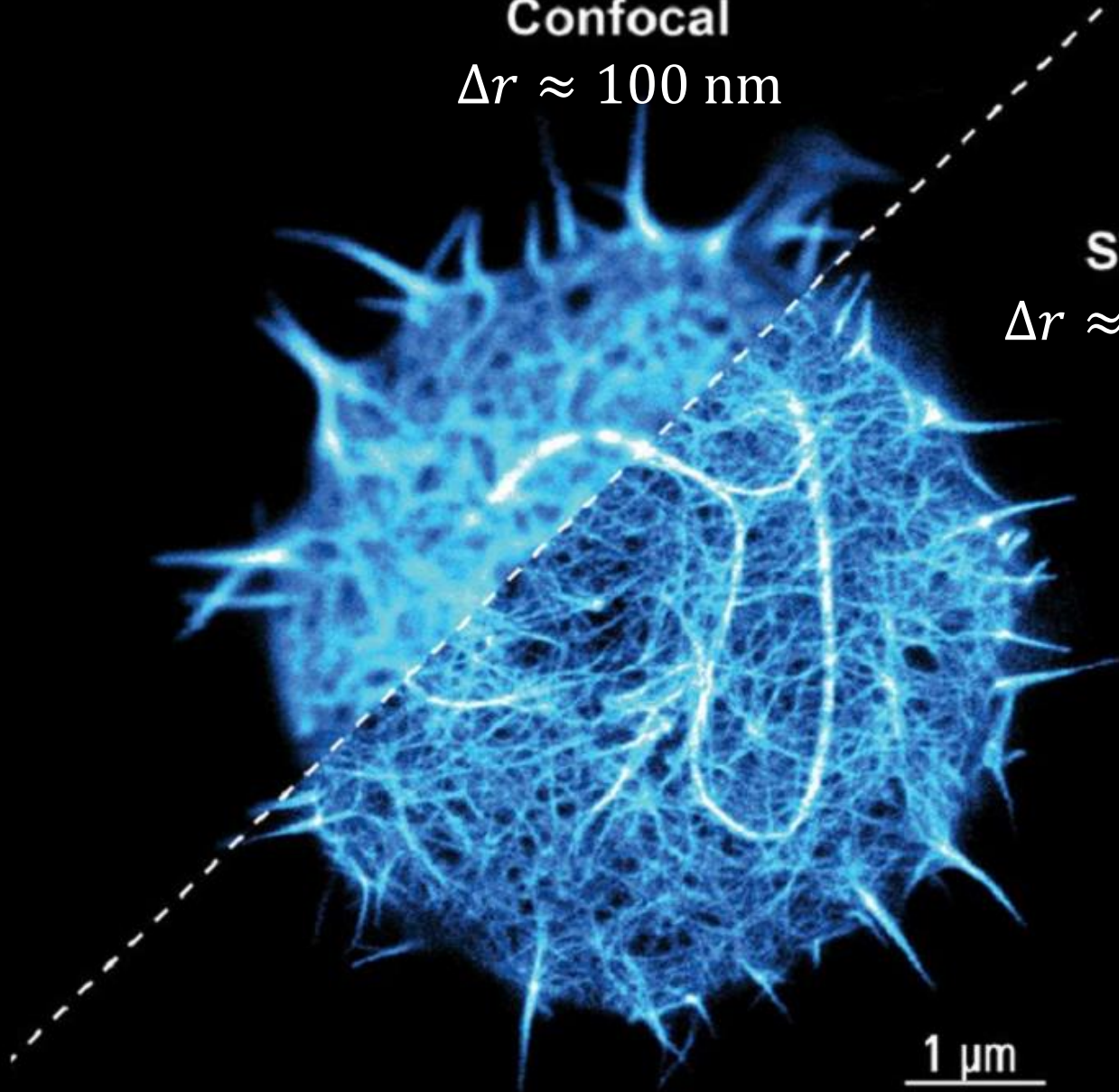
Intensité seuil

$$I_S = \frac{1}{\sigma\tau}$$



**Confocal**  
 $\Delta r \approx 100 \text{ nm}$

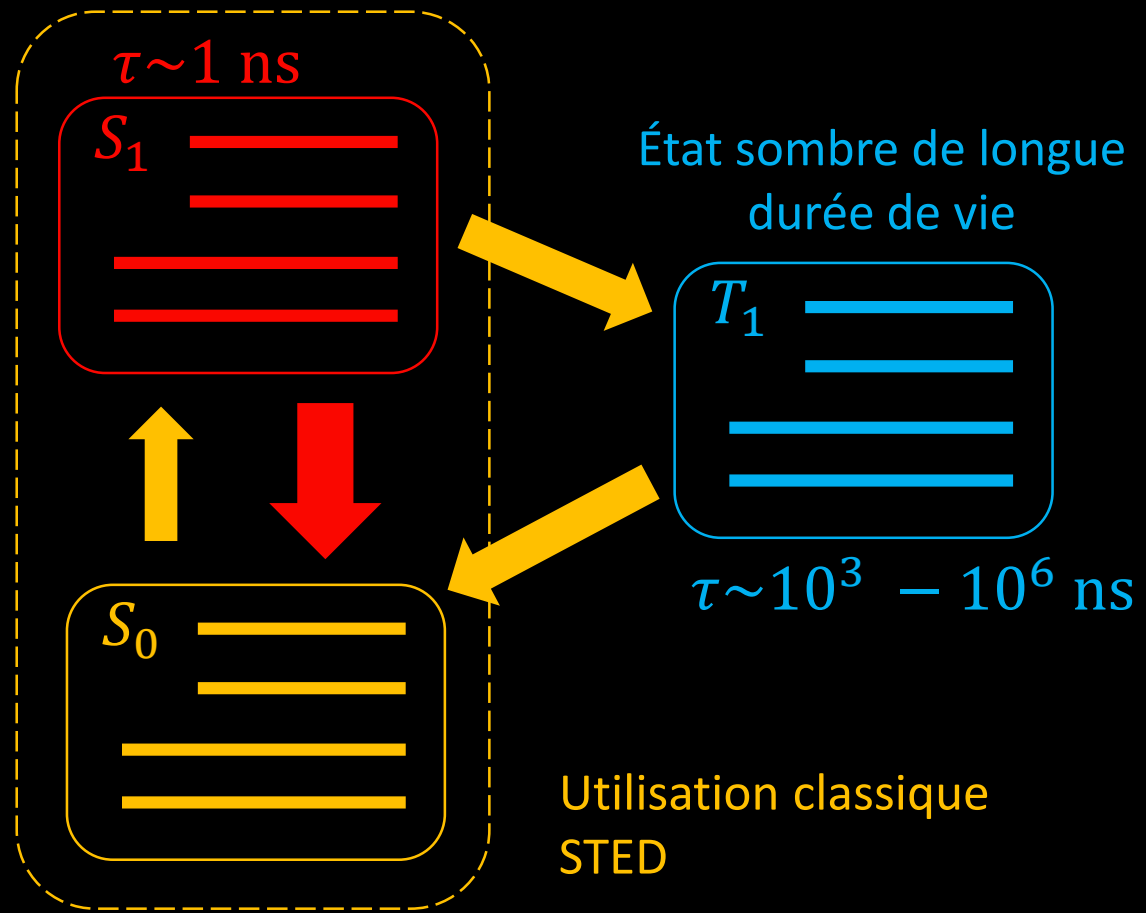
**STED**  
 $\Delta r \approx 30 \text{ nm}$



1 μm

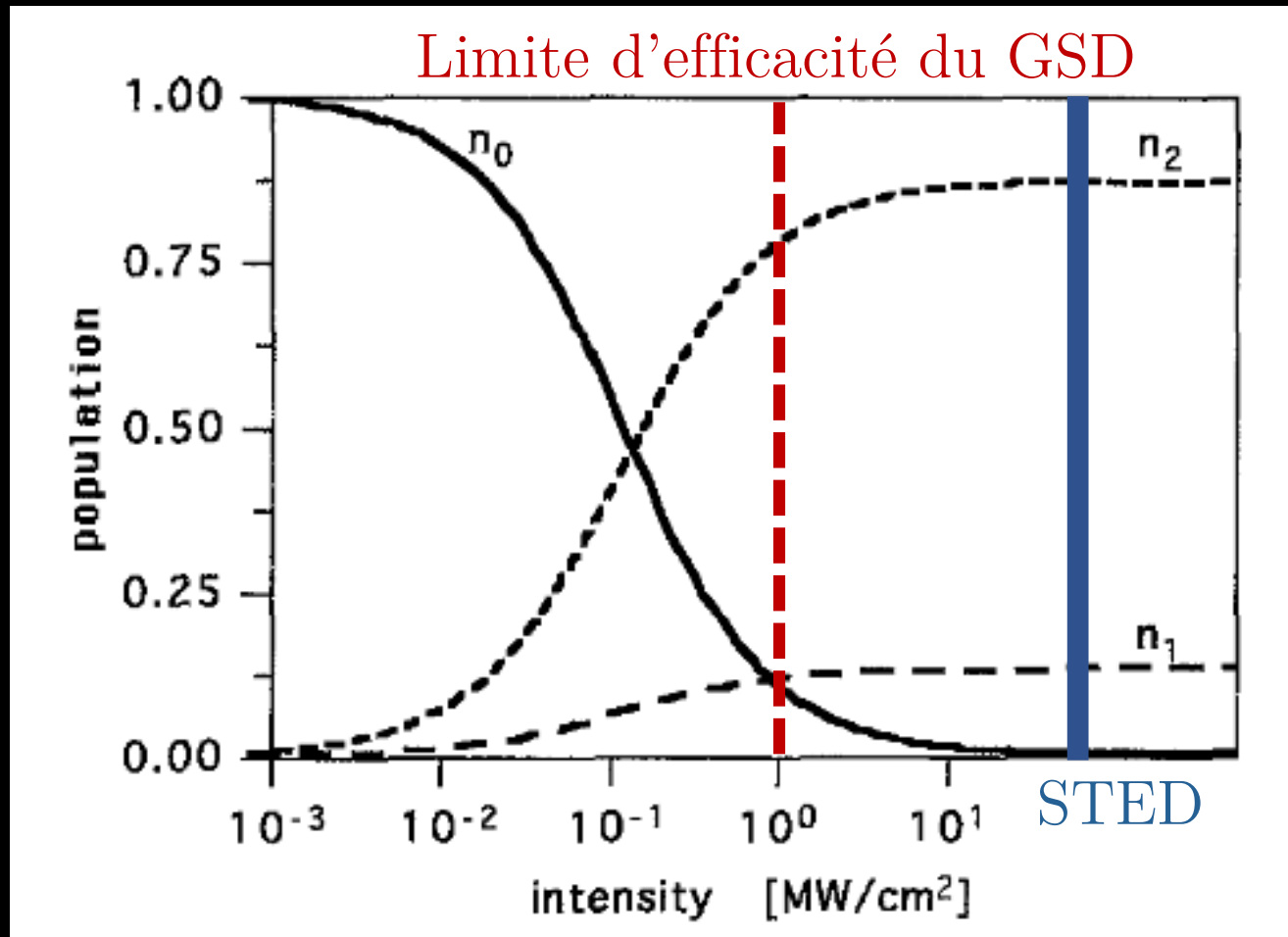
# Une variante : la microscopie GSD

Énergie



Utilisation classique  
STED

# Répartition des populations dans les différents états d'énergie en fonction de l'intensité du faisceau



$n_{0,1,2}$  : populations à l'état  $S_{0,1}$  et  $T_1$



# Une variante : la microscopie GSD

**Confocal**

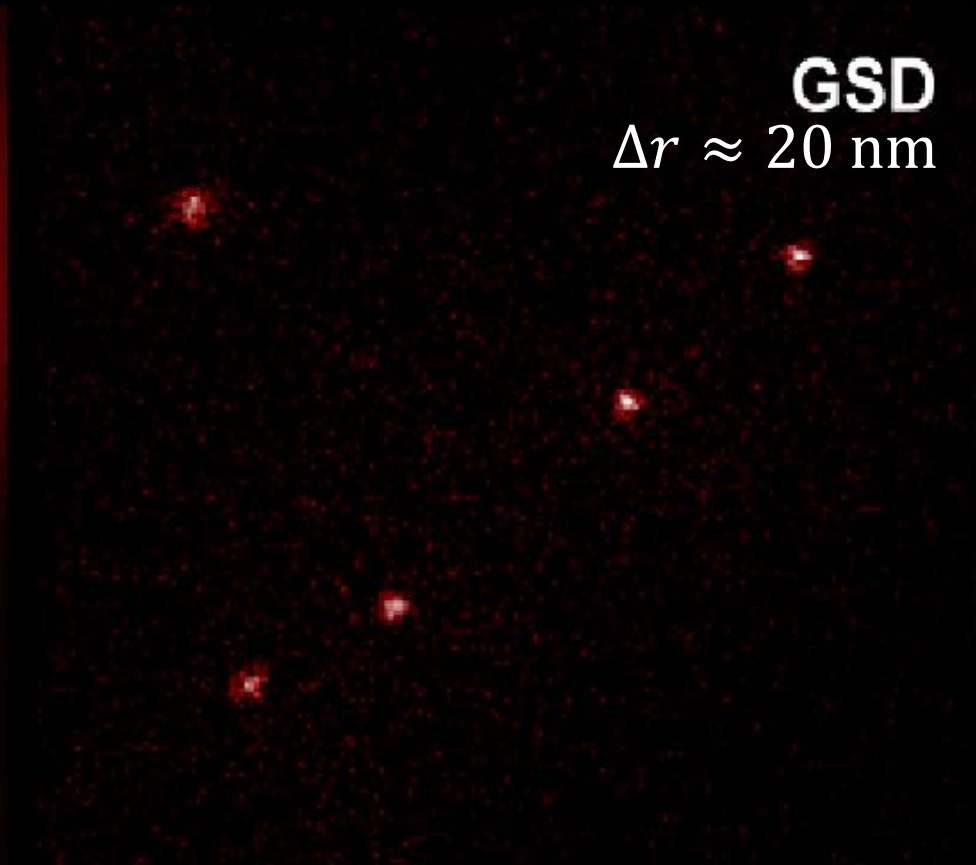
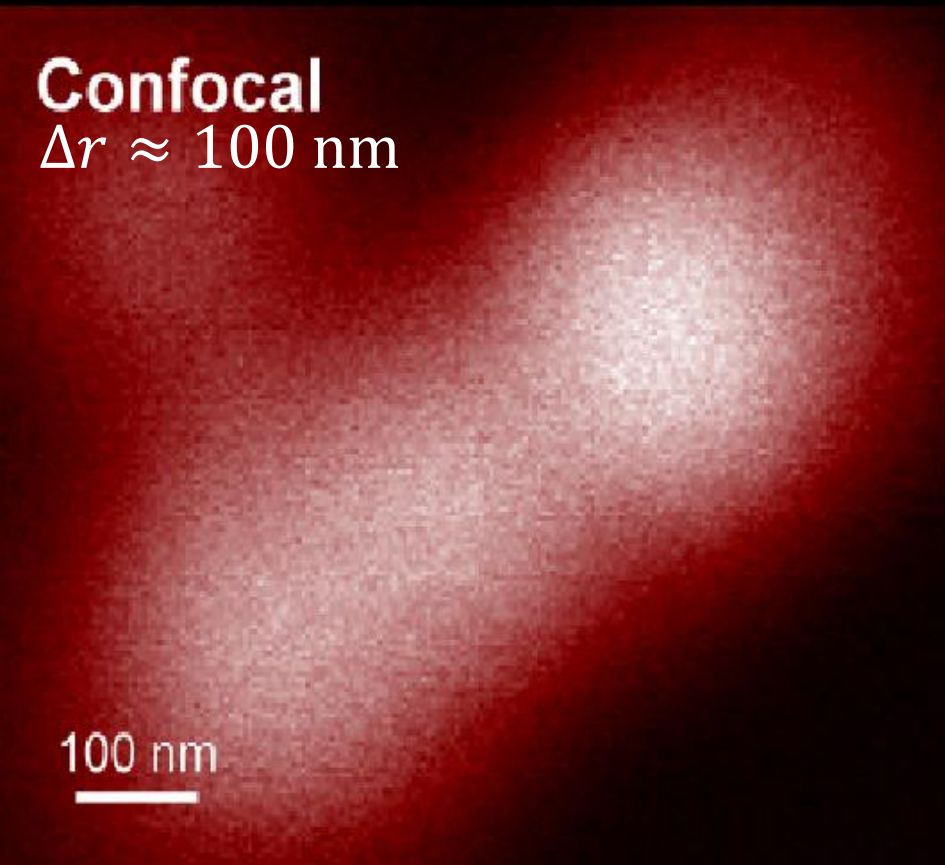
$\Delta r \approx 100 \text{ nm}$

100 nm



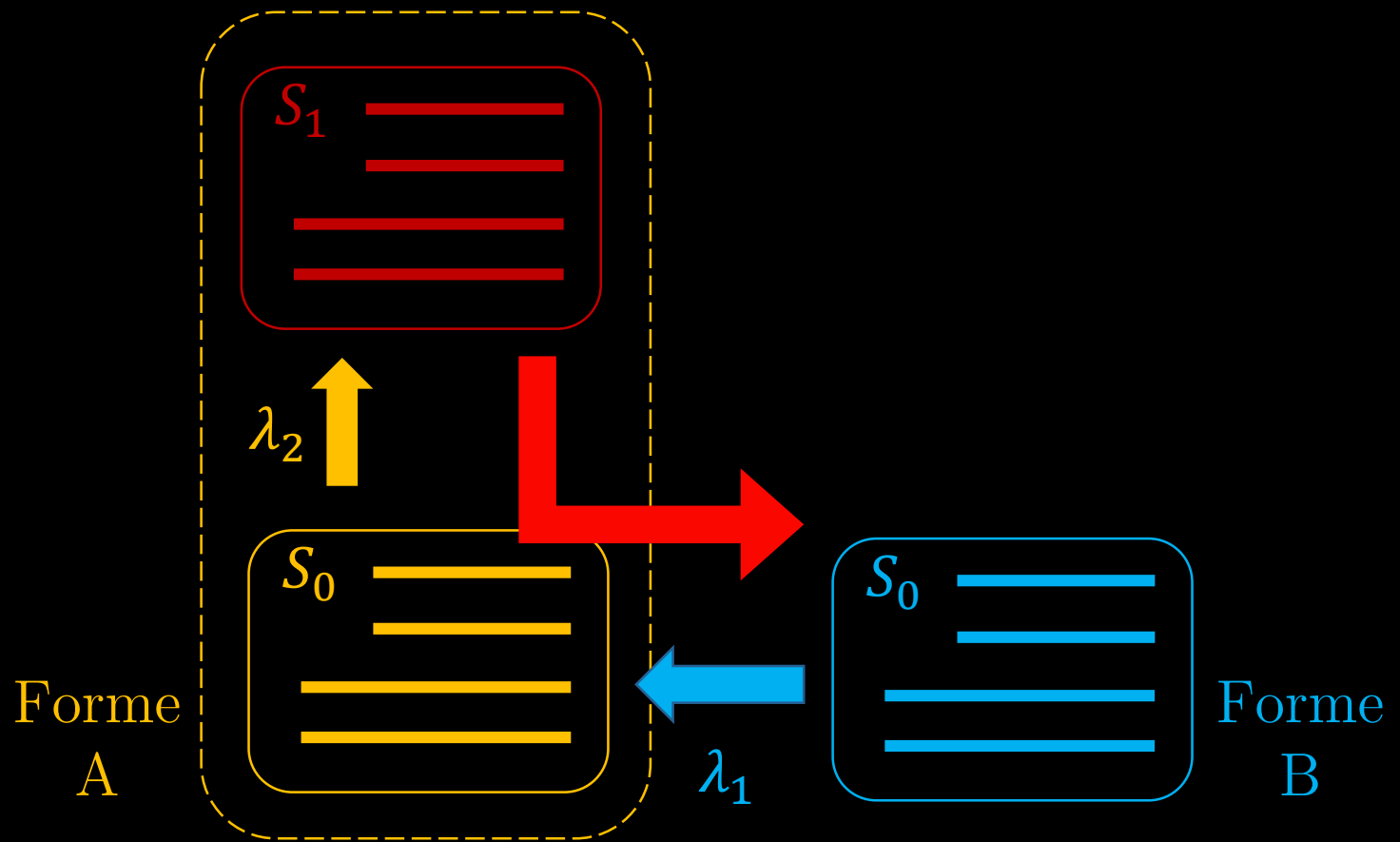
**GSD**

$\Delta r \approx 20 \text{ nm}$

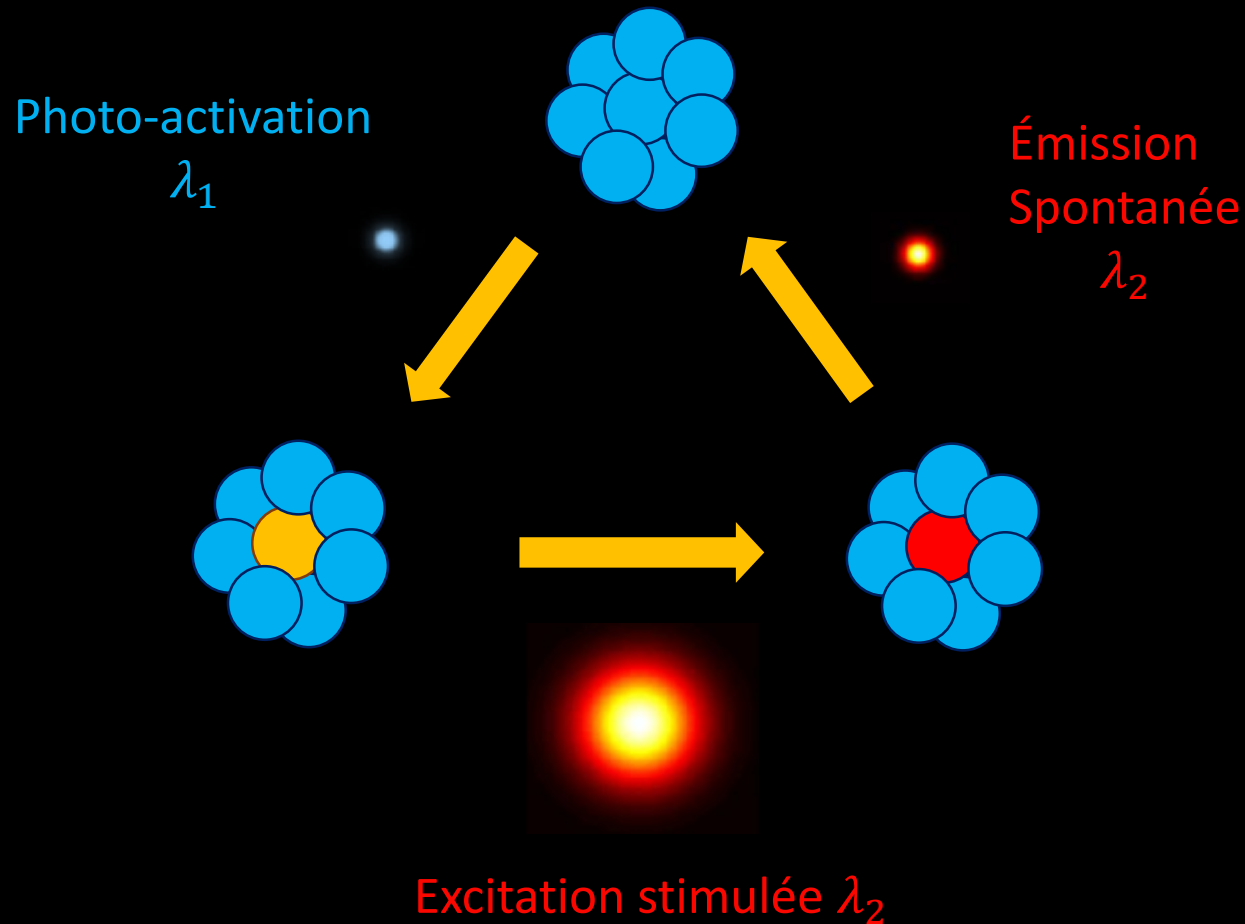


# Innovations de la technique PALM

Énergie

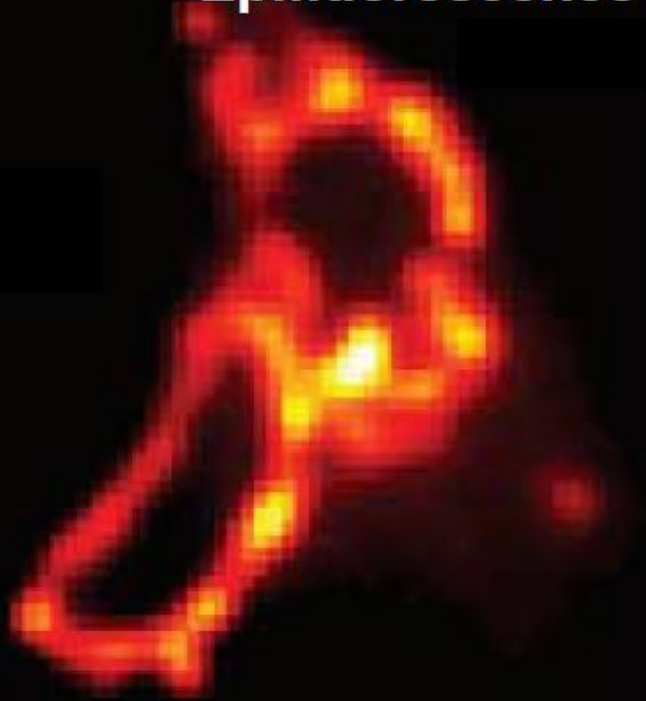


# Innovations de la technique PALM



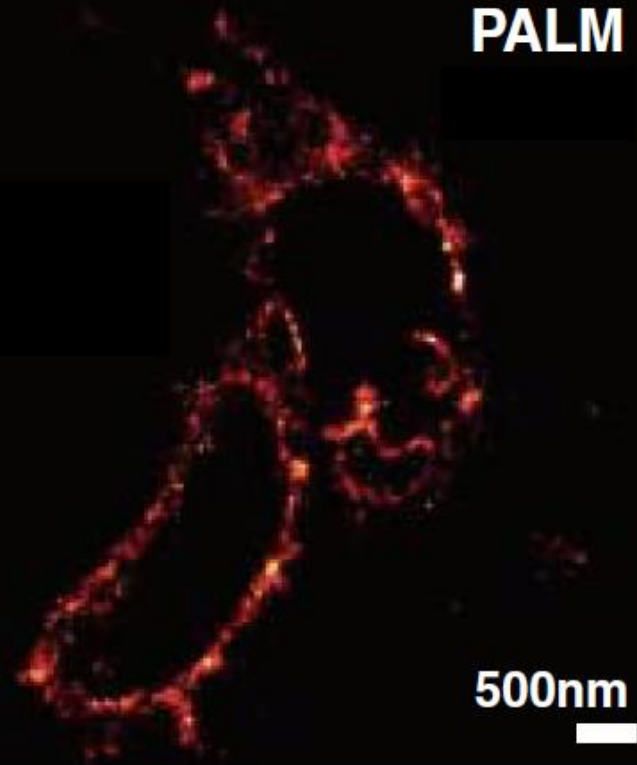
# Innovations de la technique PALM

**Epifluorescence**



$\Delta r \approx 60 \text{ nm}$

**PALM**



$\Delta r \approx 2 \text{ nm}$

# Conclusion

Diffraction



Isolement des marqueurs



STED :

Émission stimulée

GSD :

État sombre

PALM :

Conformations  
chimiques différentes

# Sources

Far-field optical nanoscopy. S.W. Hell

Leica microsystems :

<https://www.leica-microsystems.com>

Ground-State-Depletion fluorescence microscopy : a concept for breaking the diffraction resolution limit.

S.W. Hell – M. Kroug

Far-field fluorescence nanoscopy of diamond color centers by GSD

Eva Rittweger