

# **Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission**

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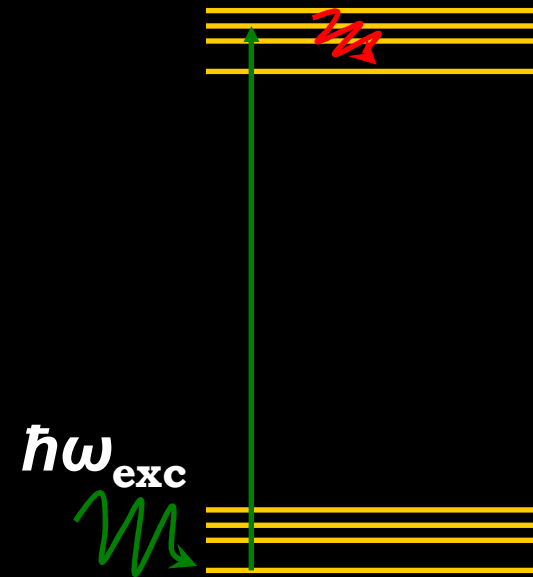
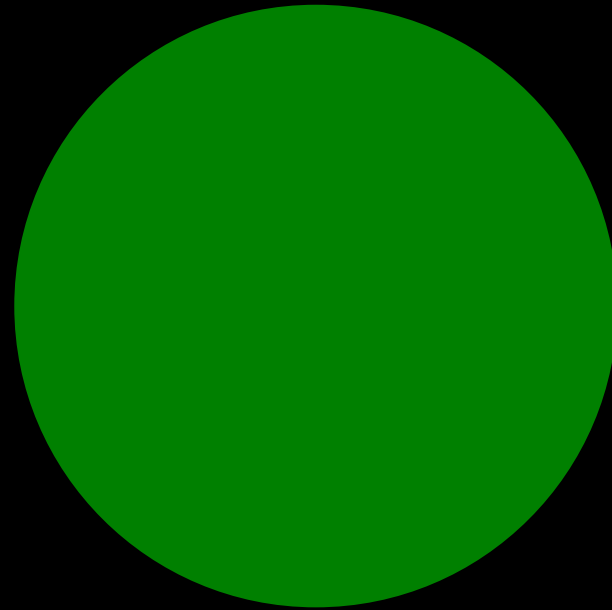
*Leandro Marcelo Acuña*

## **Límite de Abbe**

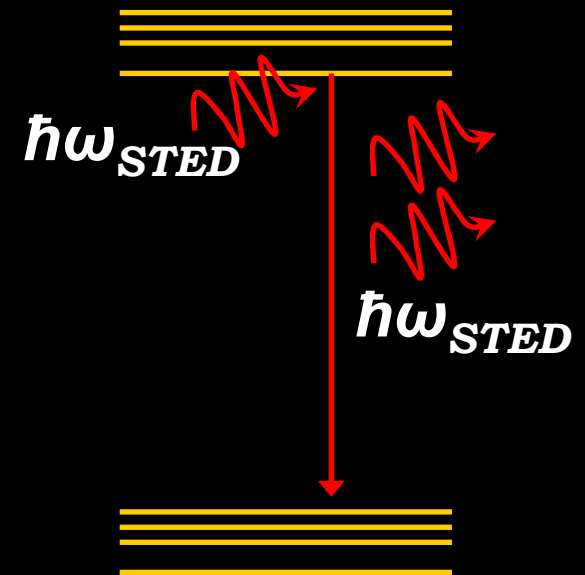
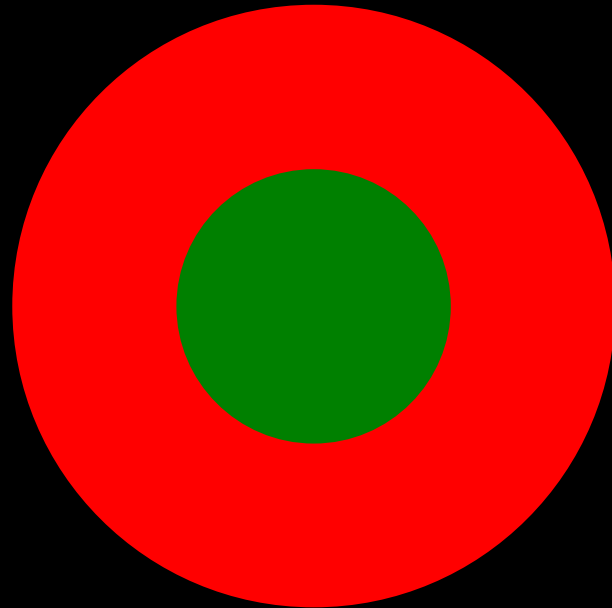
$$d = \frac{\lambda}{2n \operatorname{sen} \alpha}$$

**~ 500 – 300 nm  
en el microscopio confocal**

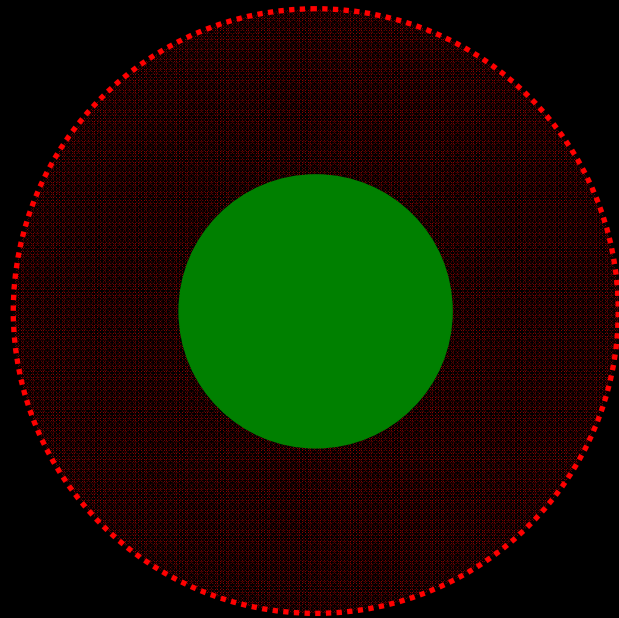
# Principios Físicos



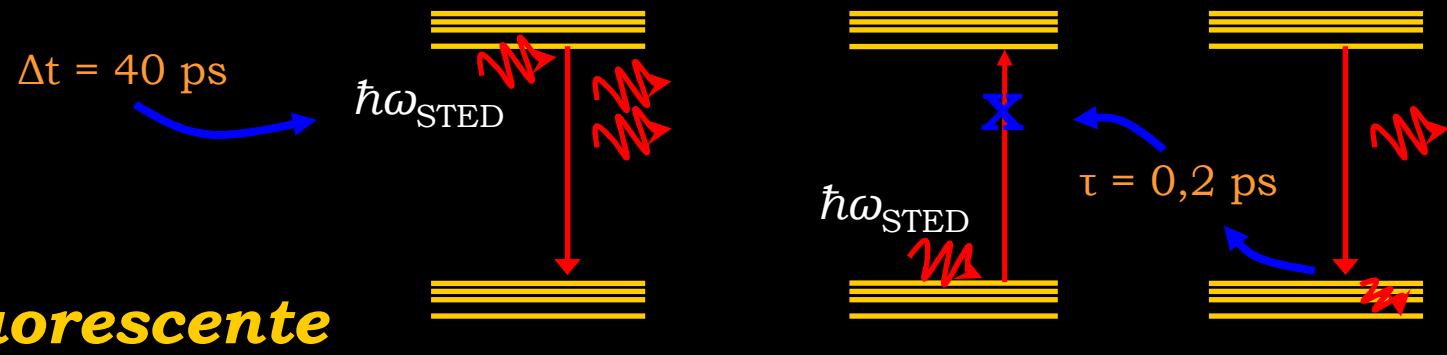
# Principios Físicos



# Principios Físicos



# Cambio temporal de las poblaciones



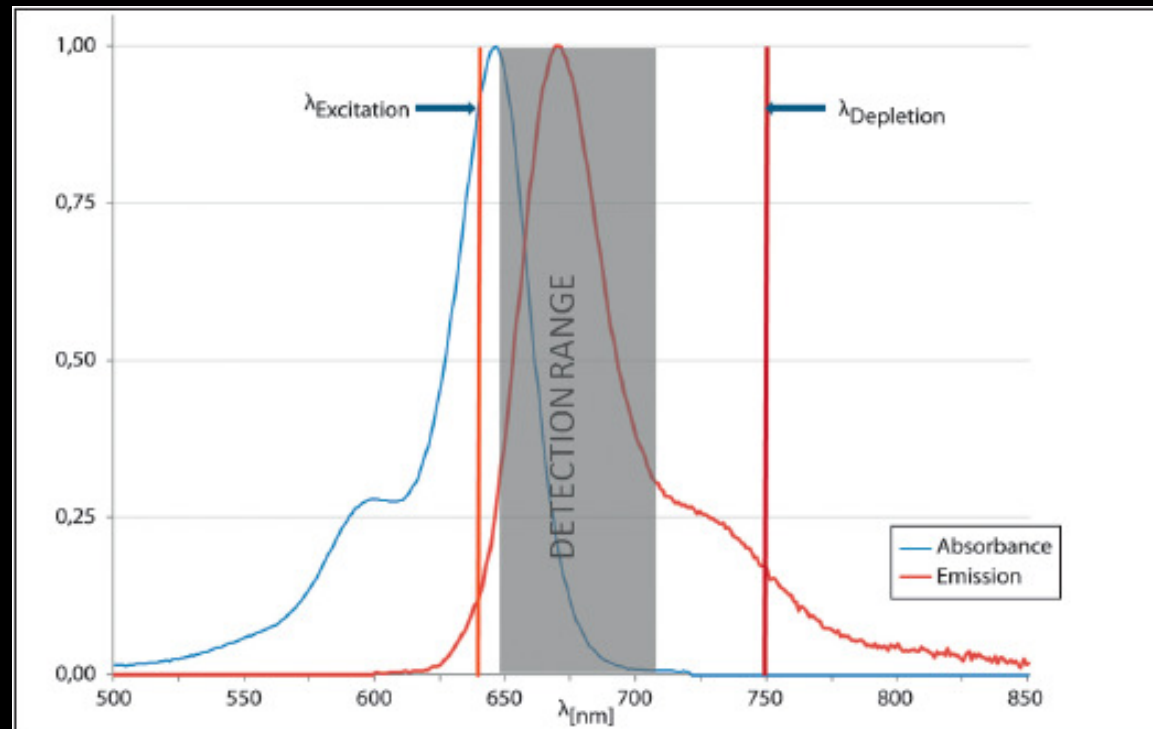
**Estado fluorescente**

$$dN_1/dt = -N_1\sigma I_{\text{STED}} / \hbar\omega_{\text{STED}} + N_0^* \sigma I_{\text{STED}} / \hbar\omega_{\text{STED}} - N_1 k_{\text{F1}} \quad [1]$$

**Nivel vibracional del estado fundamental**

$$dN_0^*/dt = N_1\sigma I_{\text{STED}} / \hbar\omega_{\text{STED}} - N_0^* \sigma I_{\text{STED}} / \hbar\omega_{\text{STED}} - N_0^* k_{\text{vib}} \quad [2]$$

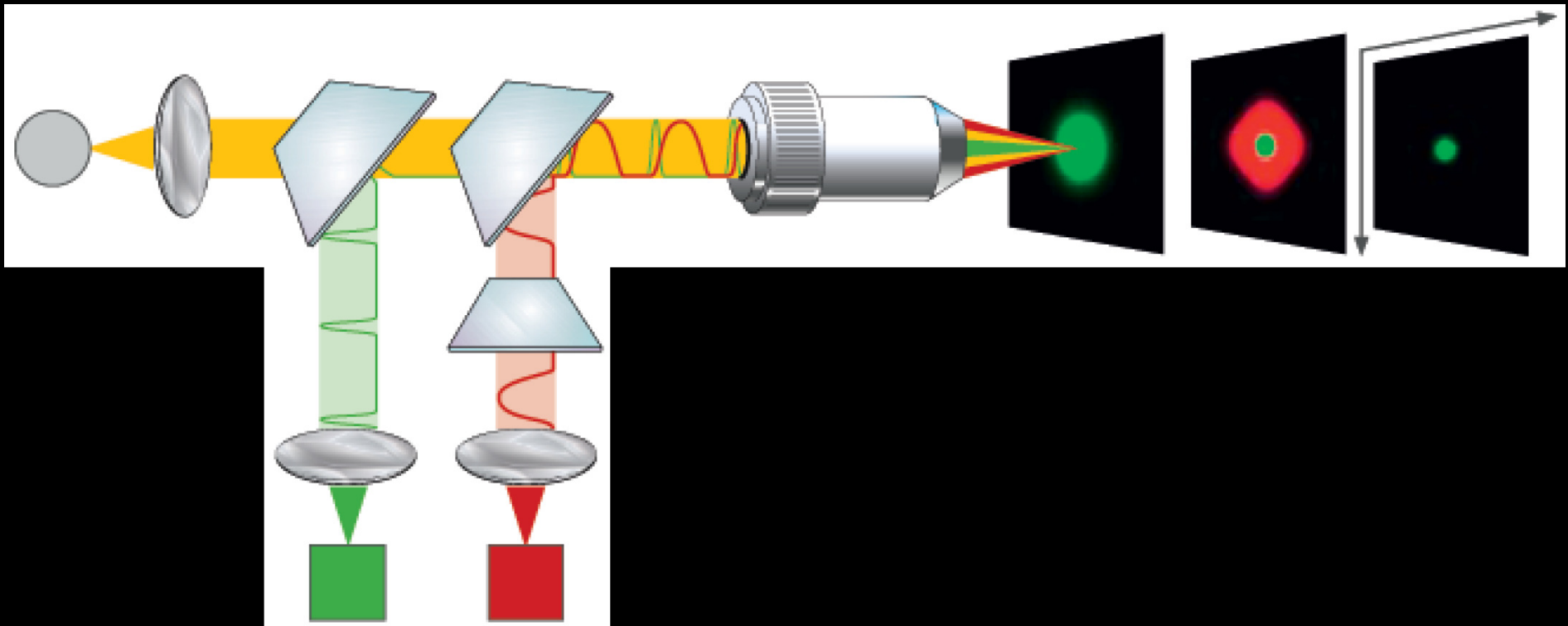
# Diseño experimental



**Espectros normalizados de absorción y emisión de ATTO 647N.  $\Delta\lambda \approx 7$  nm**

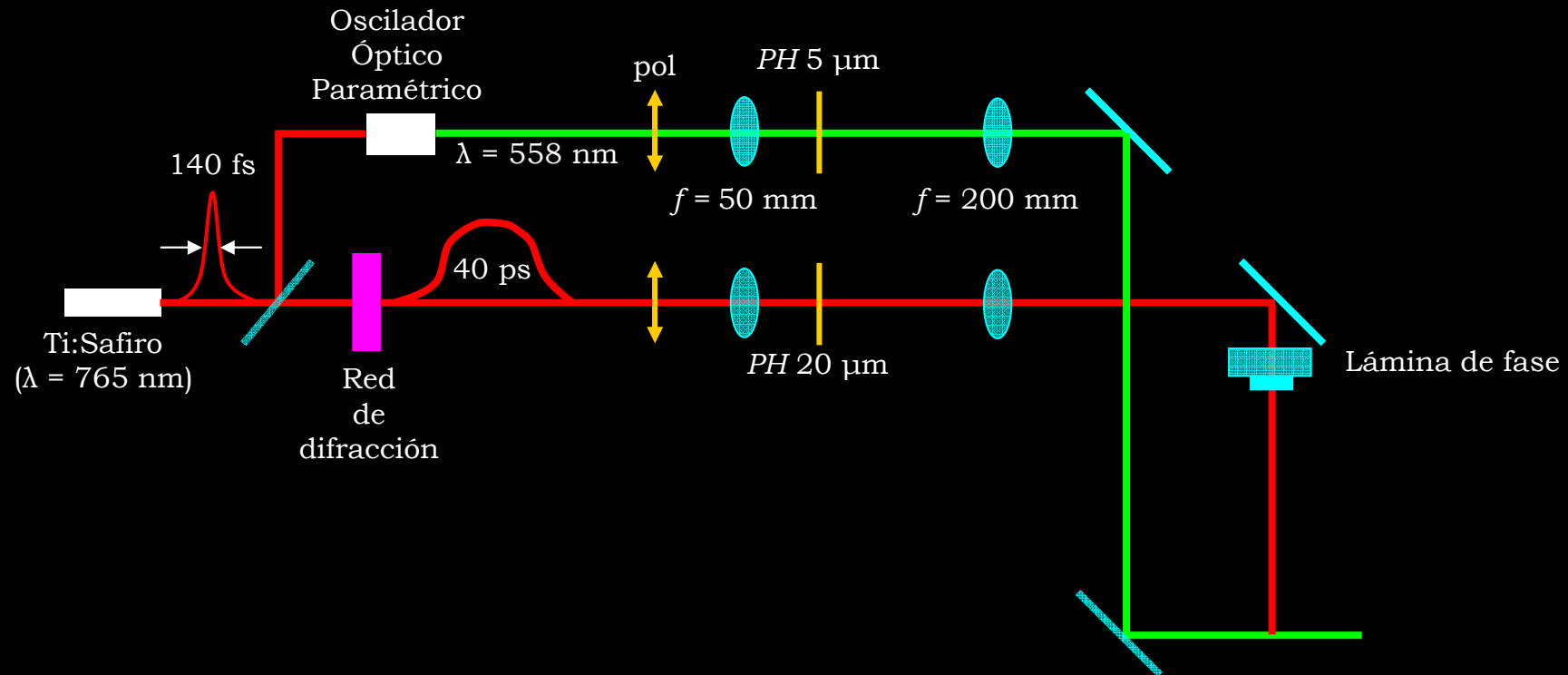
Confocal Application Letter, Feb. 2009, No. 32. Leica Microsystems. [www.leica-microsystems.com](http://www.leica-microsystems.com)

# Diseño experimental



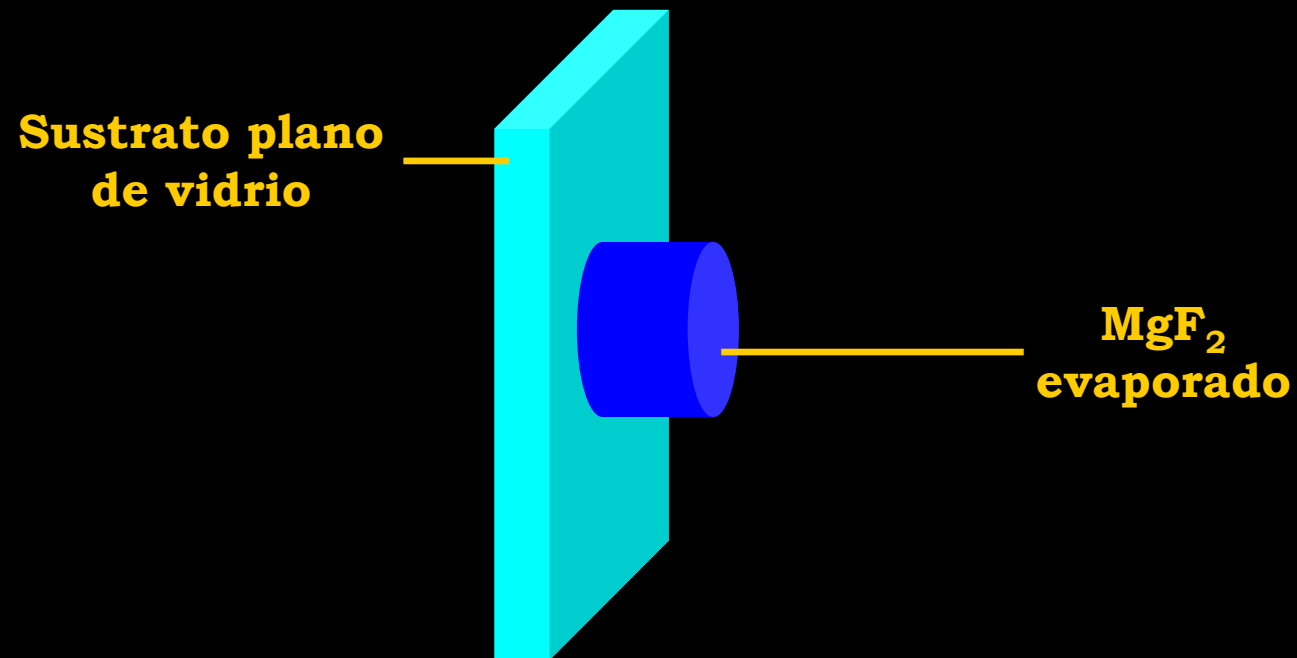


# Diseño experimental



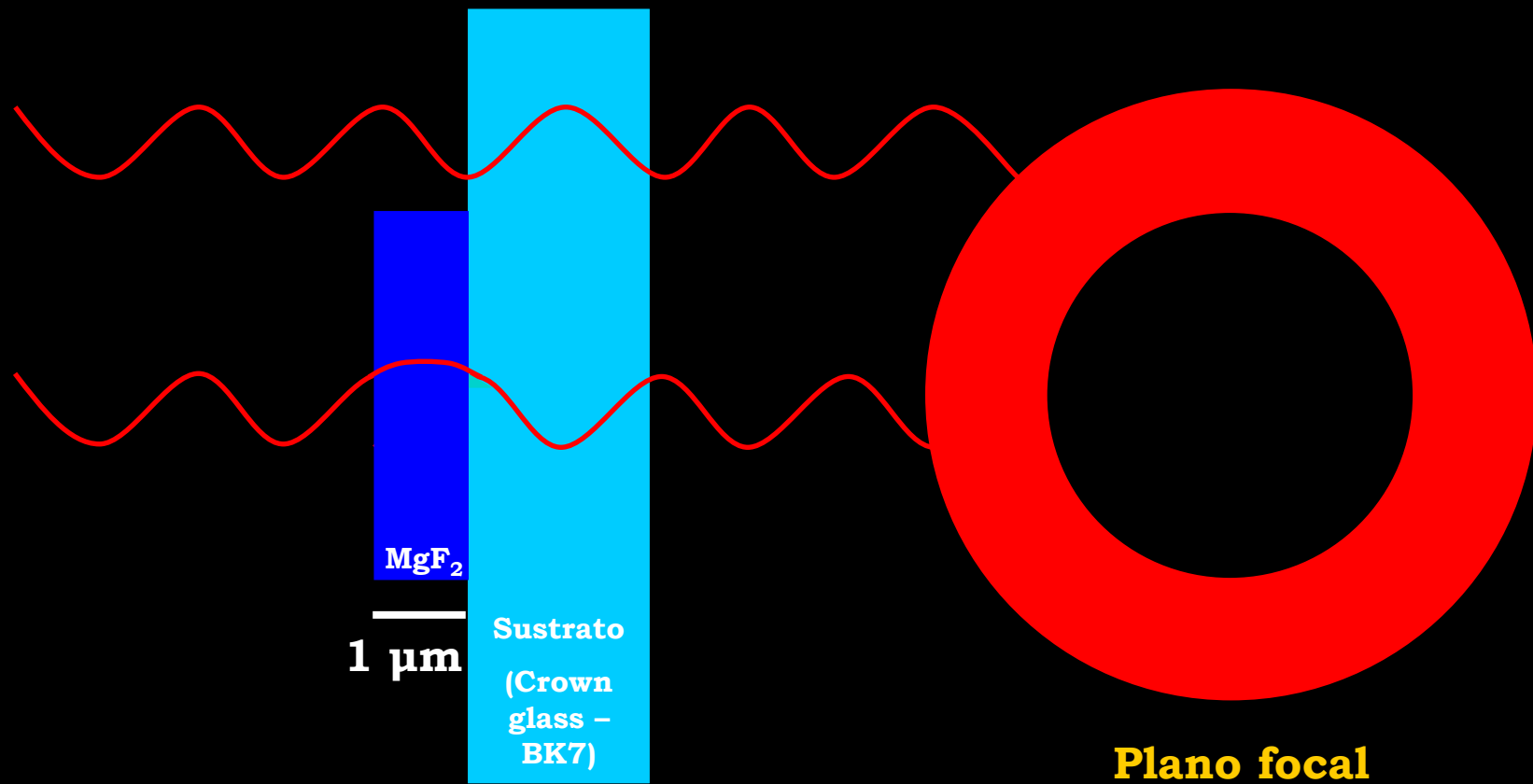
# Diseño experimental

## Optical Phase Plate

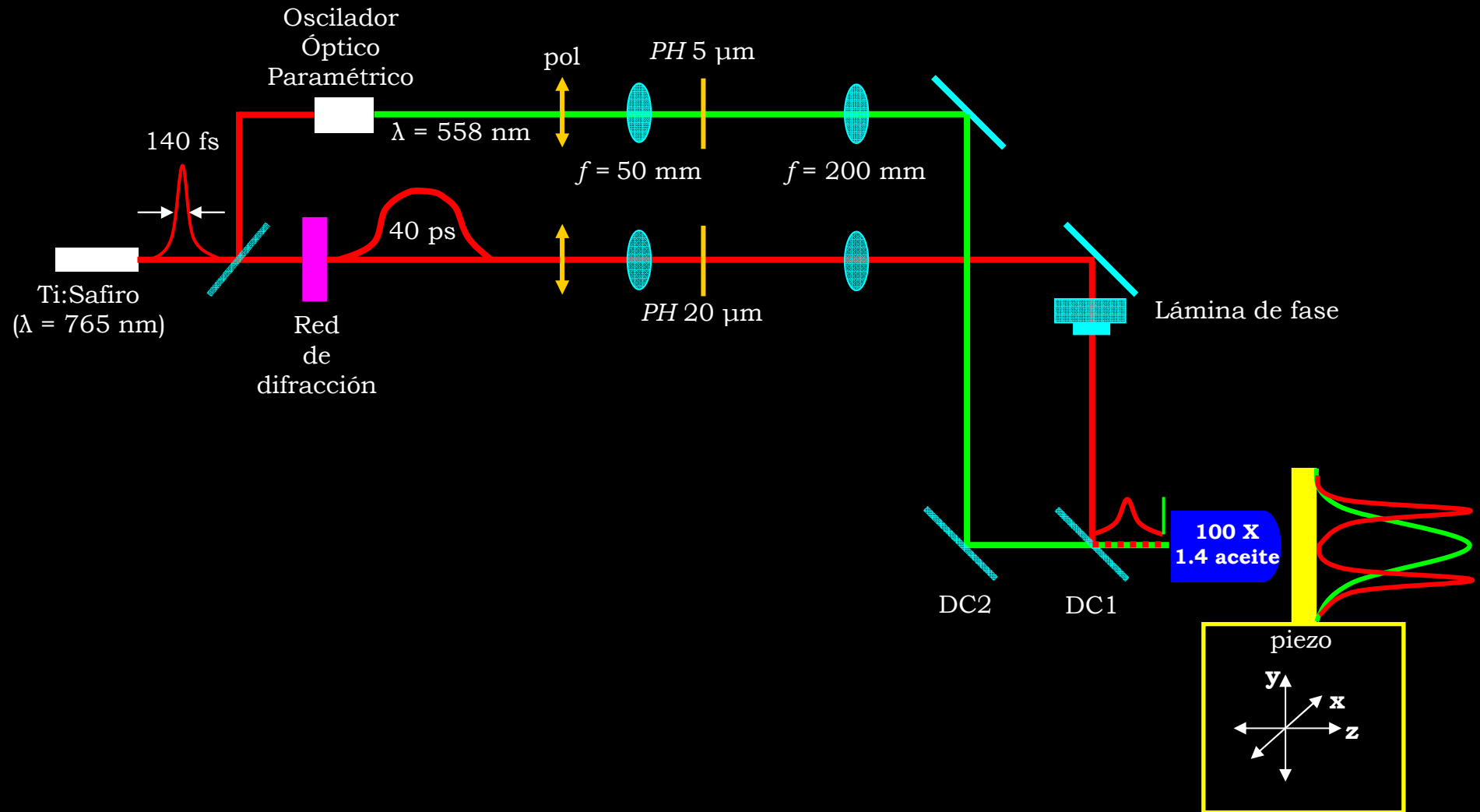


# Diseño experimental

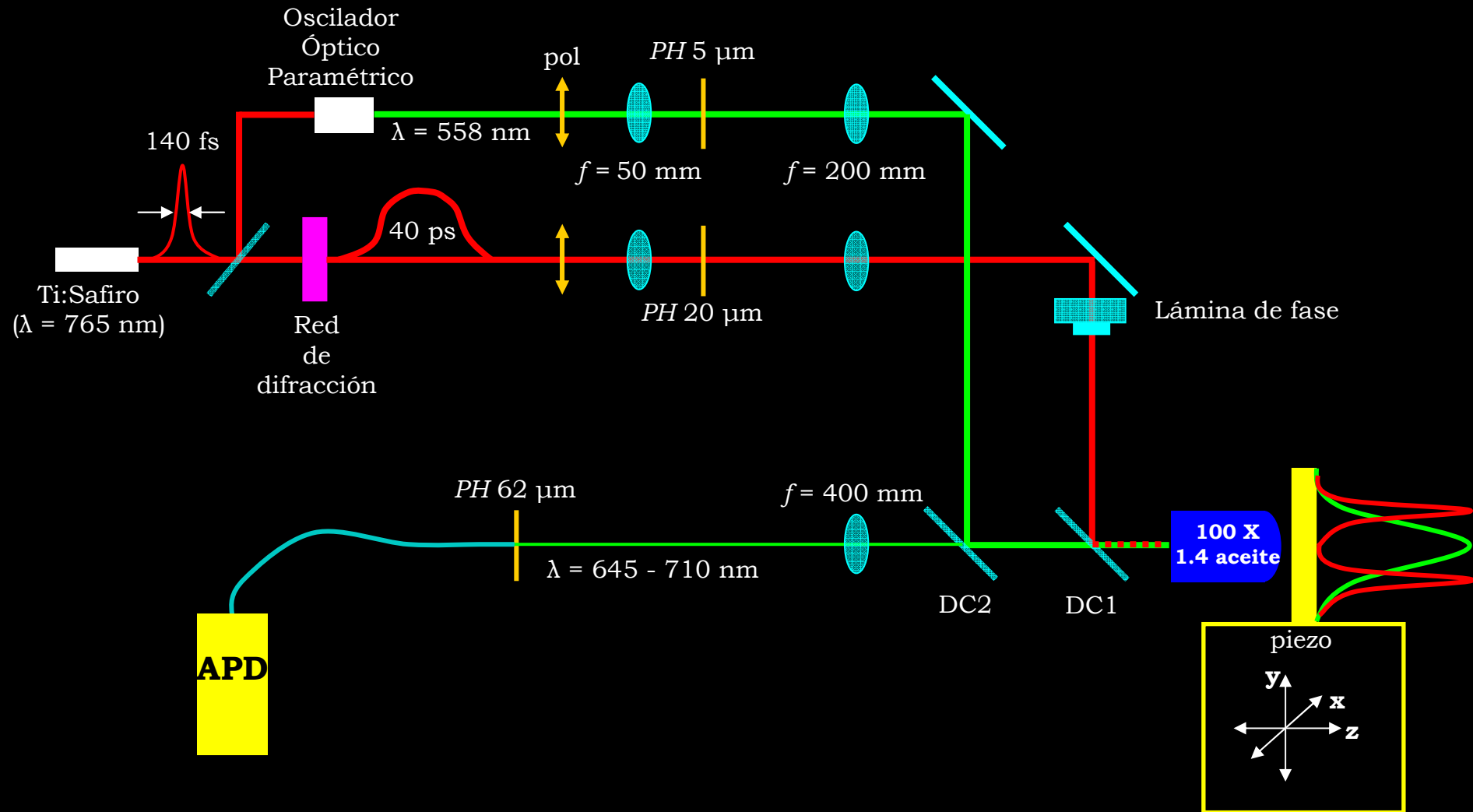
## Ingeniería del haz STED



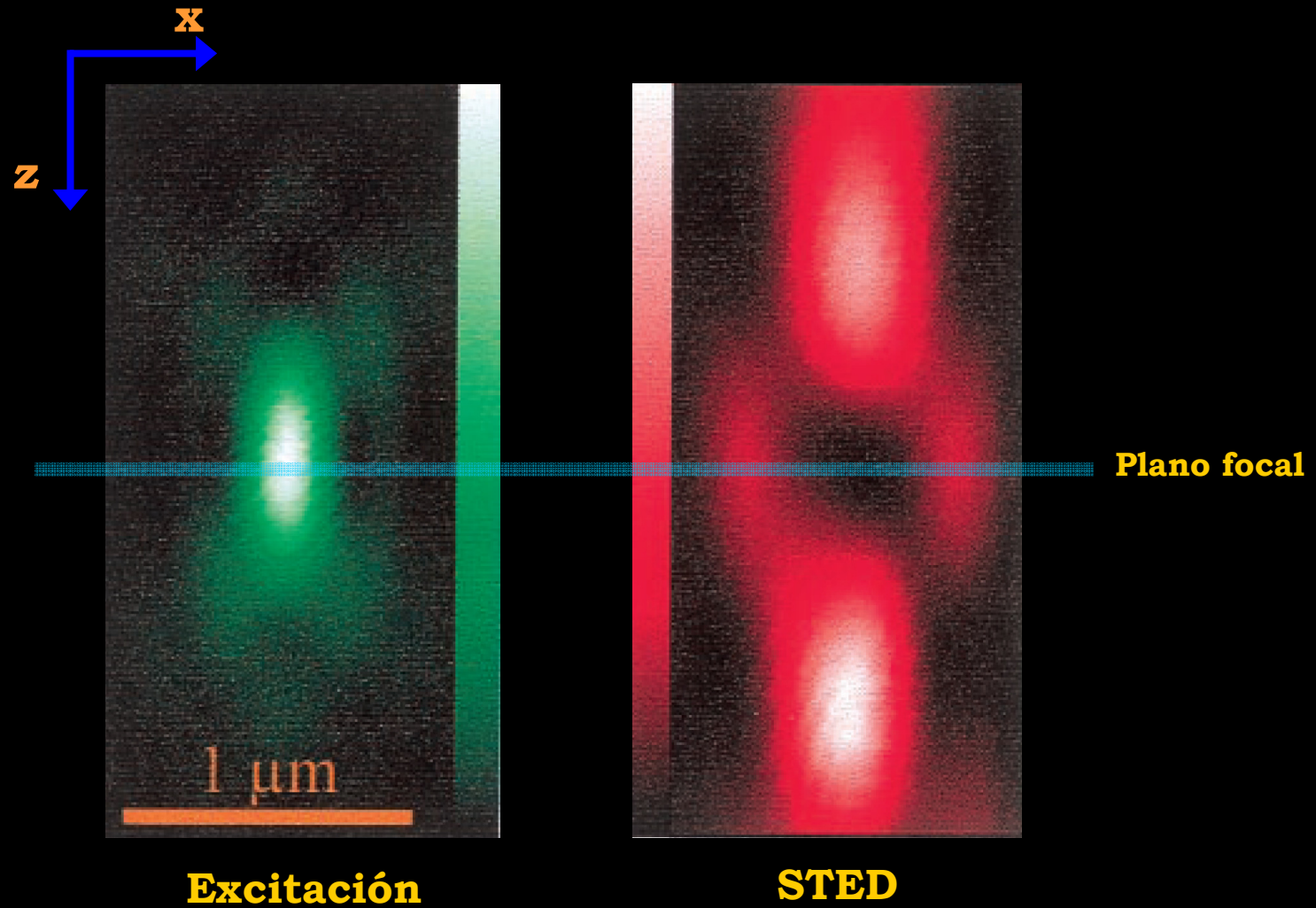
# Diseño experimental



# Diseño experimental



# Diseño experimental



# Resolución espacial en función de la intensidad del STED

$$\Delta r \approx \frac{\lambda}{2n \operatorname{sen} \alpha \sqrt{1 + I_{\max} / I_s}}$$

*Factor de supresión:*  $\eta \equiv \exp(-\ln 2 I_{\text{STED}}(x) / I_s)$

Distribución de intensidad del **STED** cerca del punto focal:

Aproximo el **E** linealmente, luego **I** puede ser aproximada por una parábola

$$I_{\text{STED}}(x) = 4 I_{\text{STED}} a^2 x^2$$

PSF efectivo del microscopio STED:

$$h_{\text{eff}}(\mathbf{x}) = h_c(\mathbf{x}) \eta(\mathbf{x}) = \exp(-4 \ln 2 x^2 (d_c^{-2} + a^2 I_{\text{STED}}(x) / I_s))$$

Resolución:

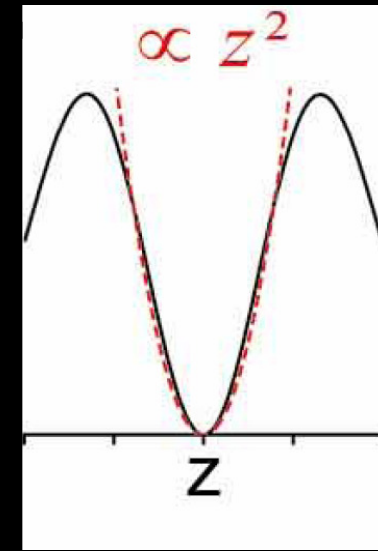
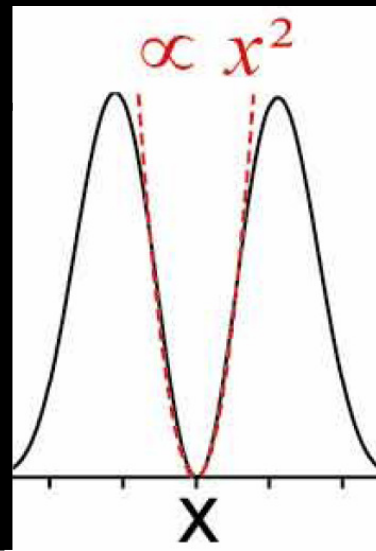
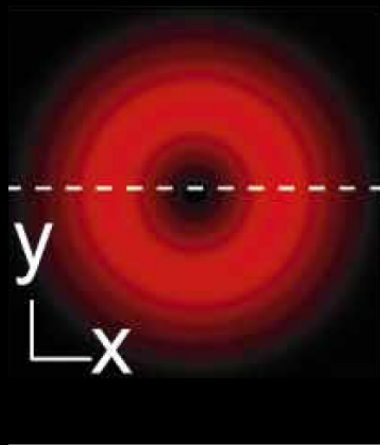
$$d = d_c / \sqrt{1 + d_c^2 a^2 I_{\text{STED}}(x) / I_s} \quad \xrightarrow{I_{\text{STED}} \gg I_s} \quad d = 1 / a \sqrt{I_{\text{STED}}(x) / I_s}$$

# Resolución espacial en función de la intensidad del STED

Distribución de intensidad del **STED** cerca del punto focal:

Aproximo el **E** linealmente, luego **I** puede ser aproximada por una parábola

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# Resultados