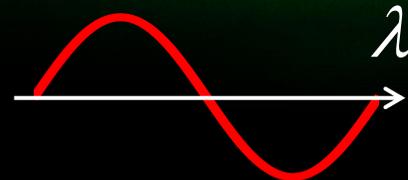
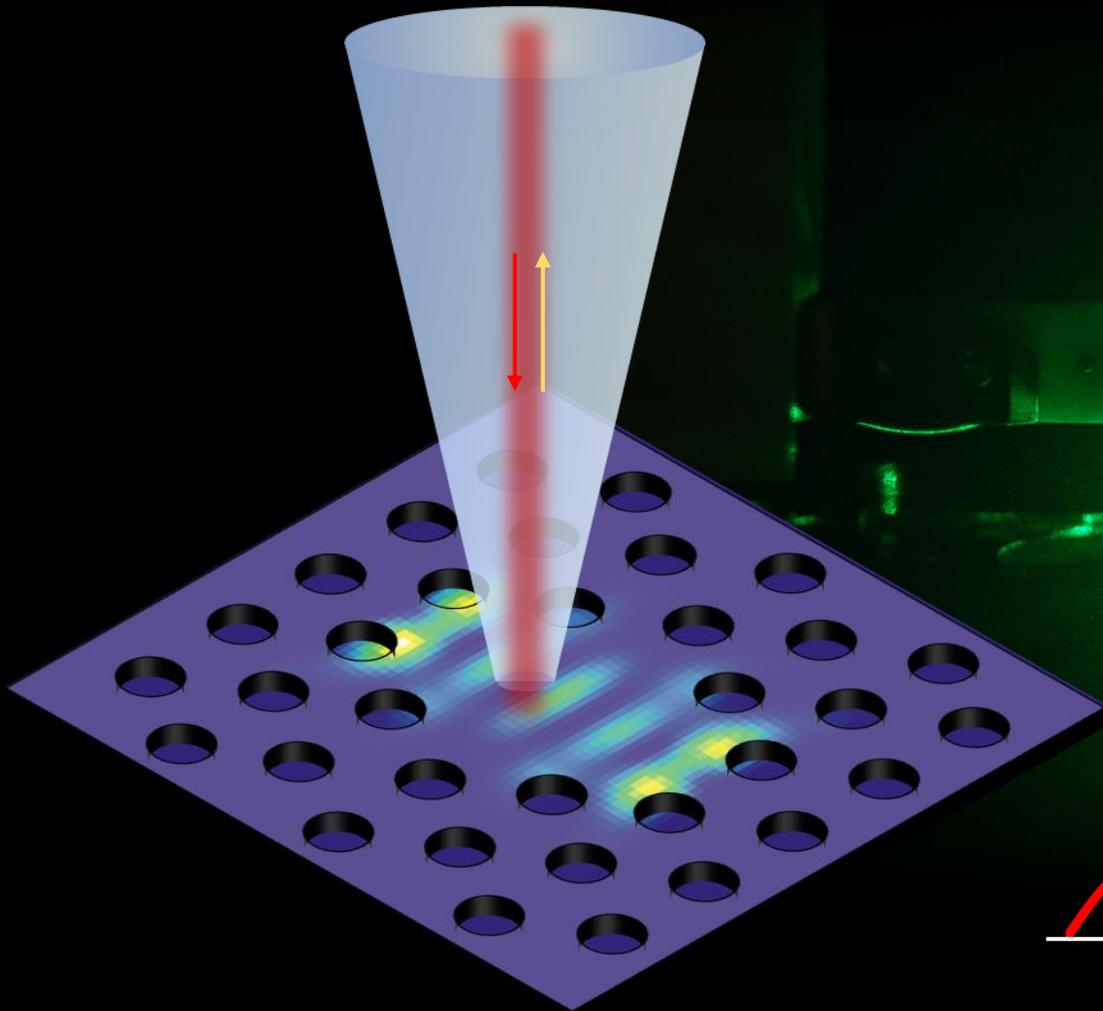


# Microscopia in Campo Prossimo

Nicoletta Granchi

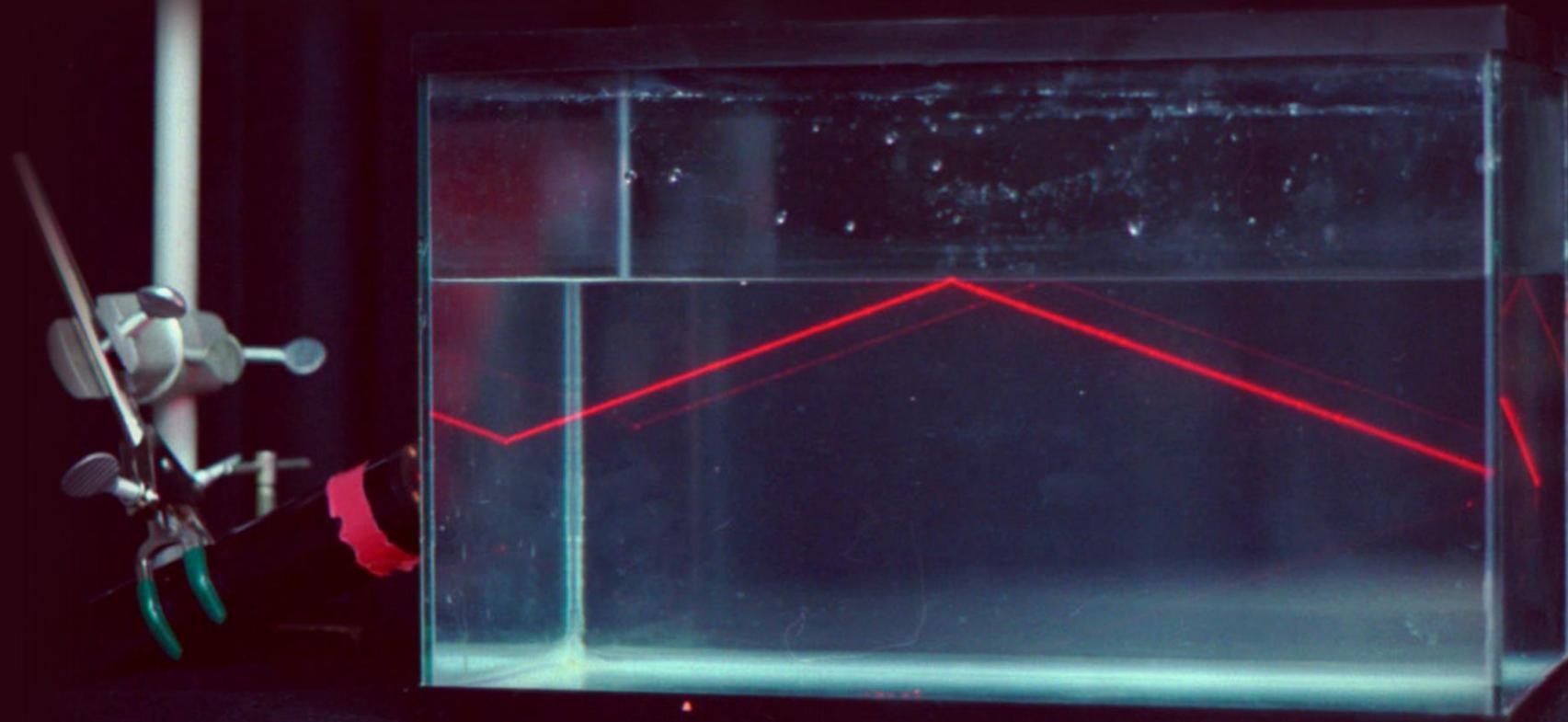


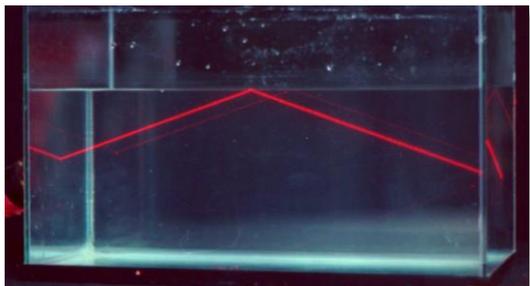
Dipartimento di Fisica e Astronomia,  
Università degli Studi di Firenze



Laboratorio Europeo per le  
Spettroscopie Non-lineari – LENS

# Riflessione totale interna

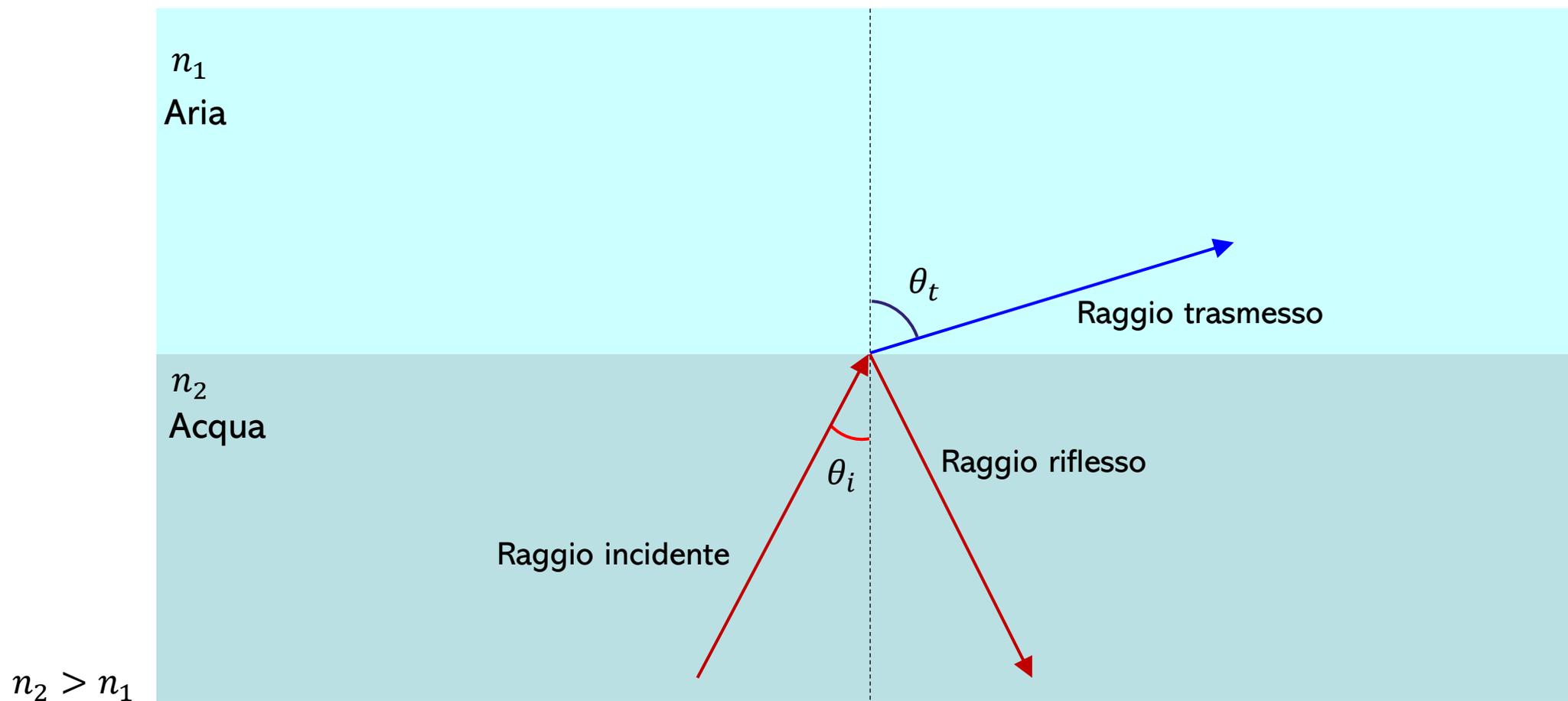


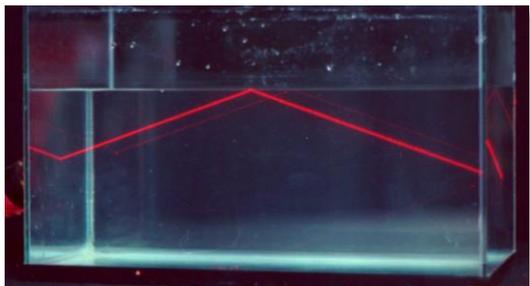


# Riflessione Totale Interna

Legge di Snell

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

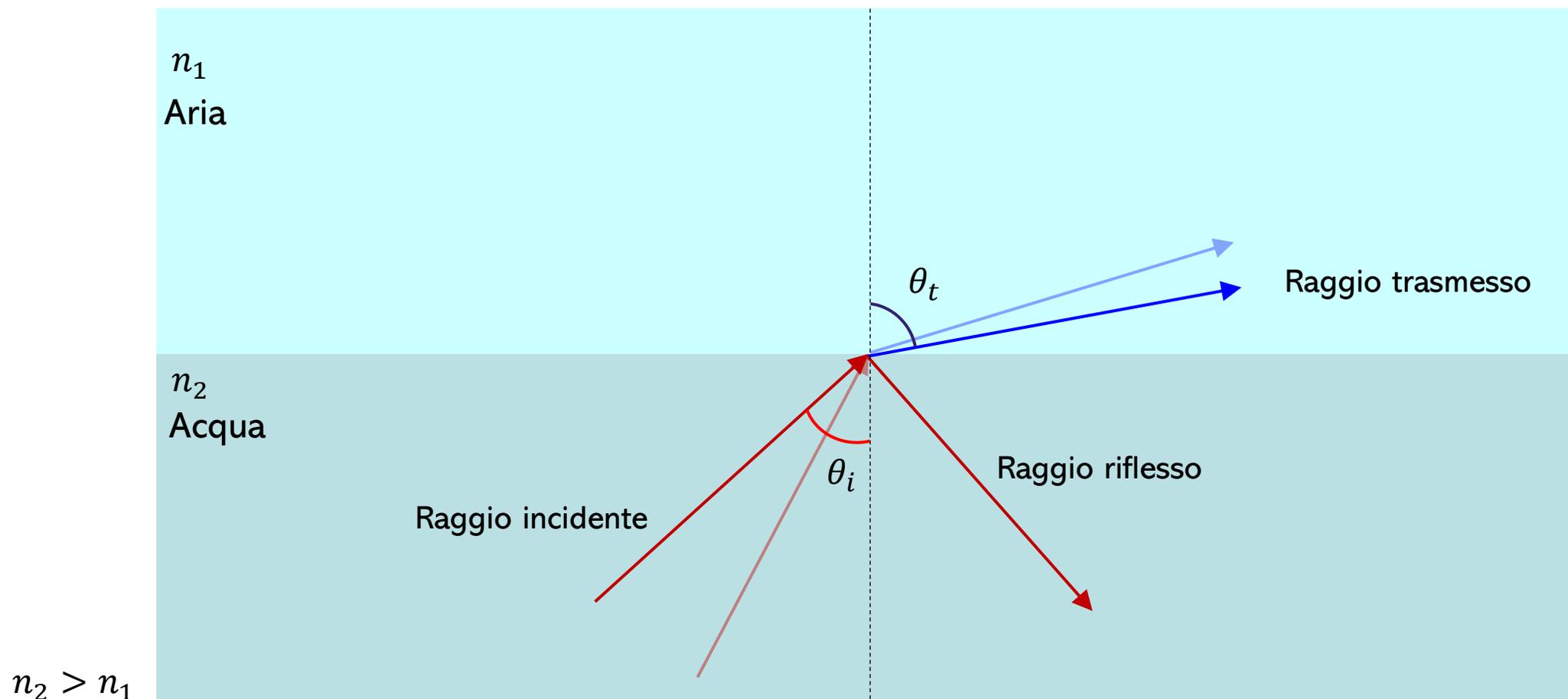


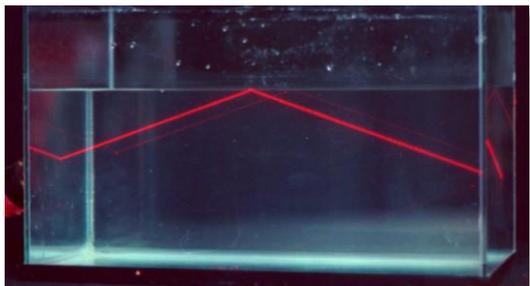


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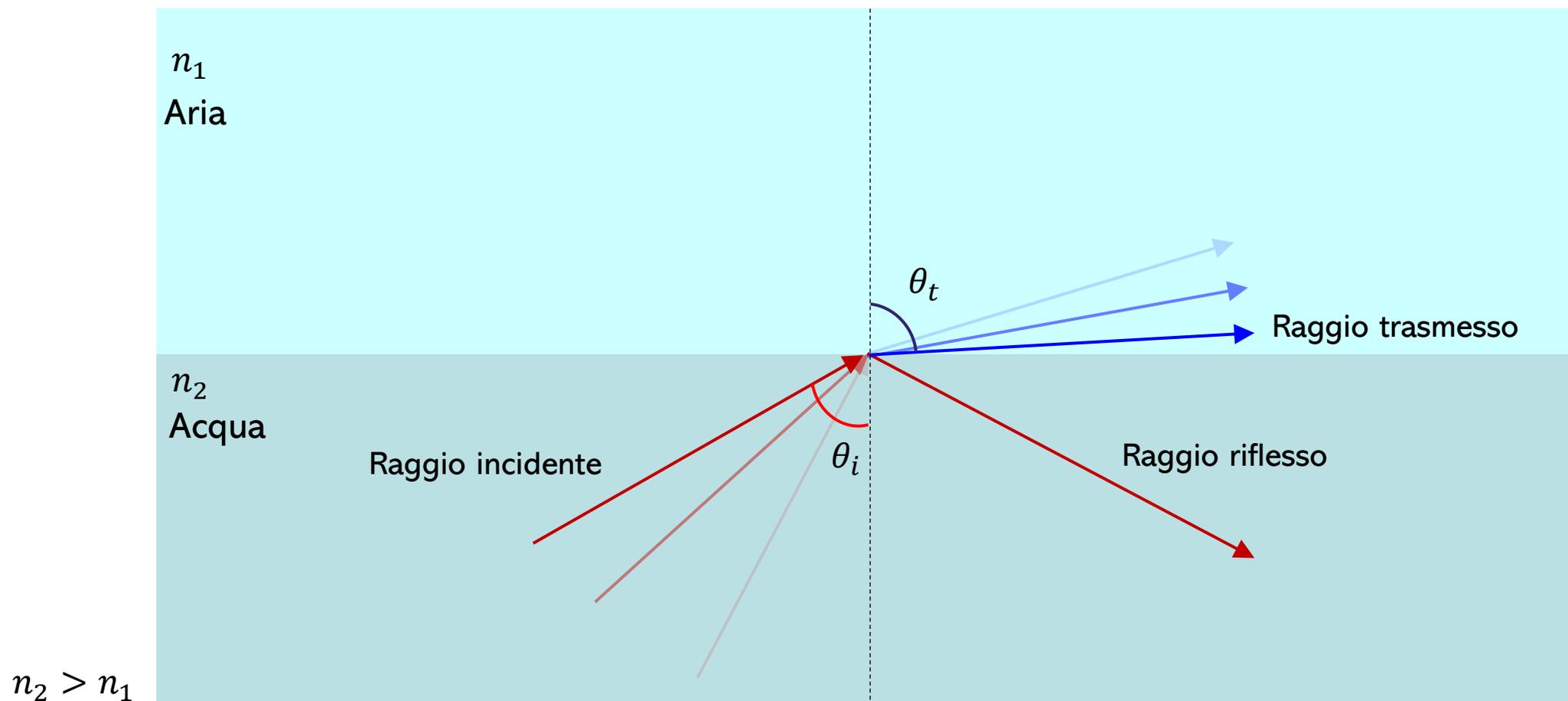


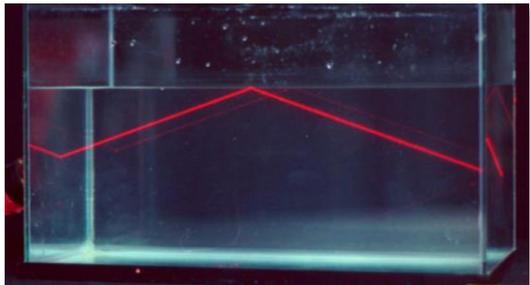


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Legge di Snell

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

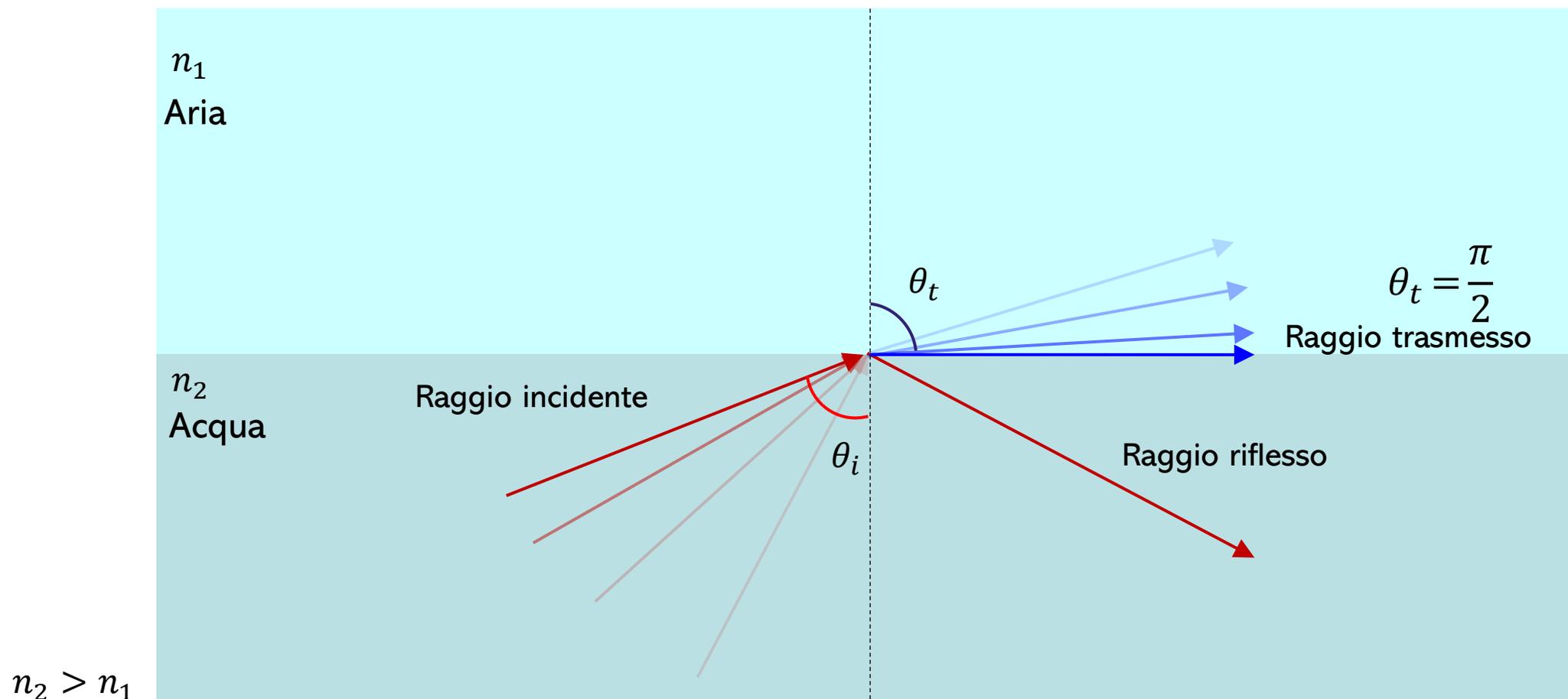


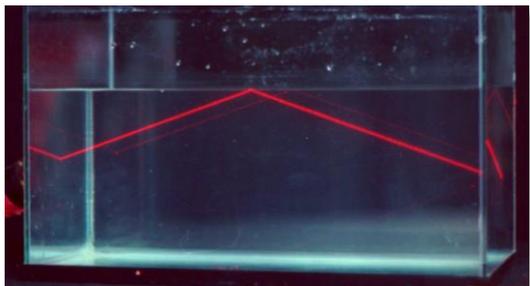


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$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

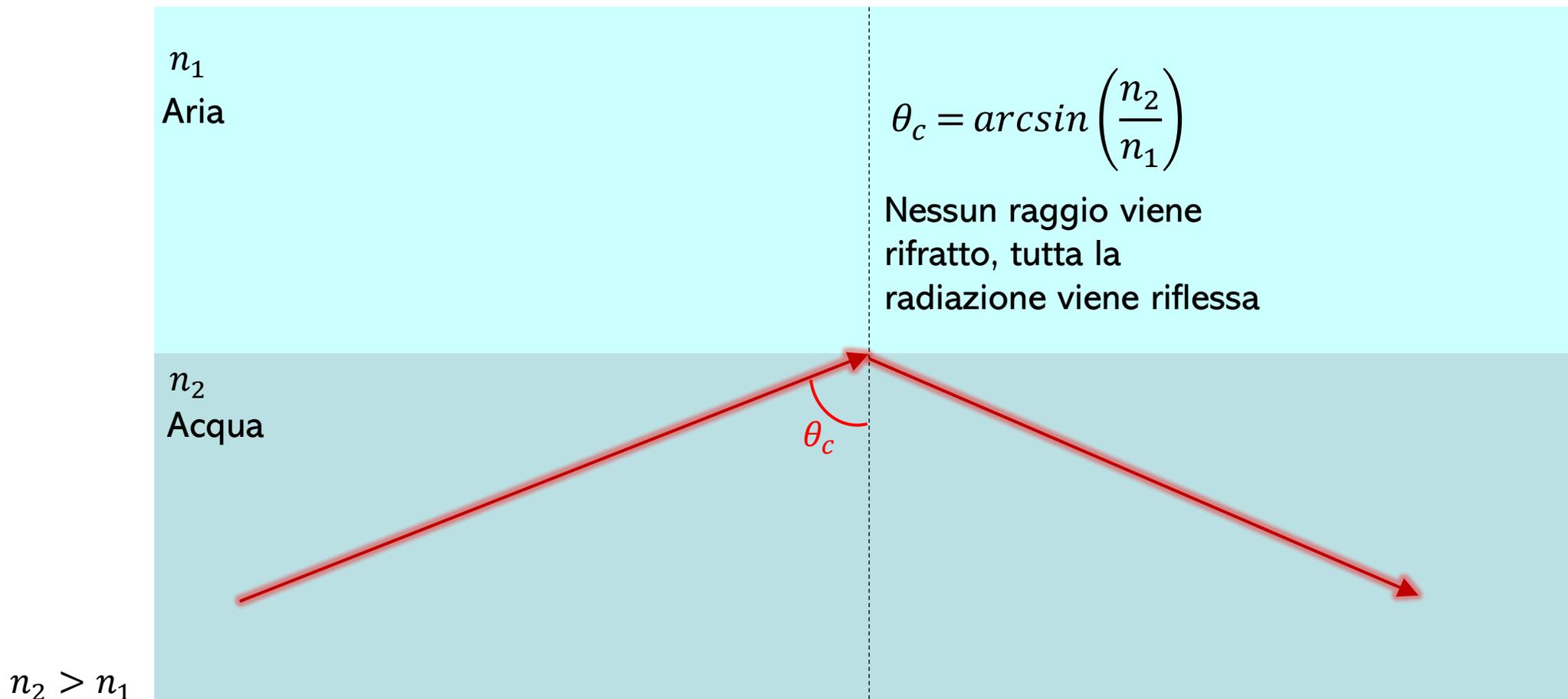


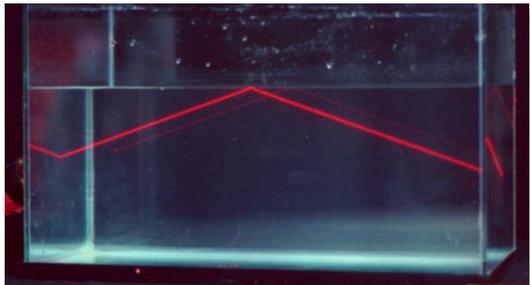


# Riflessione Totale Interna

Legge di Snell

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

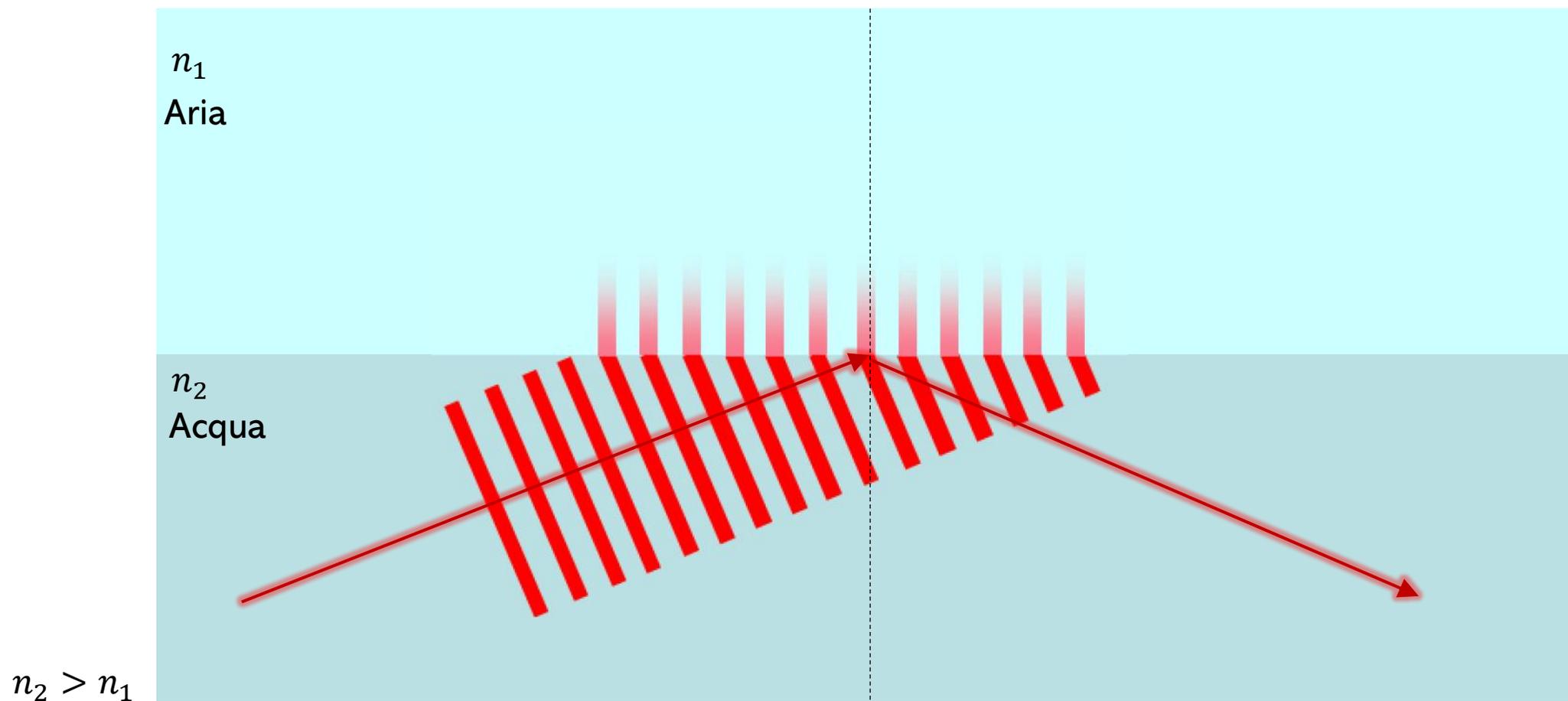


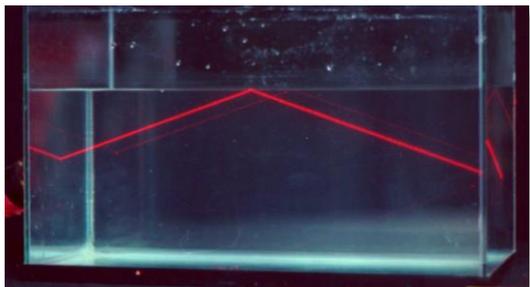


# Riflessione Totale Interna

Legge di Snell

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

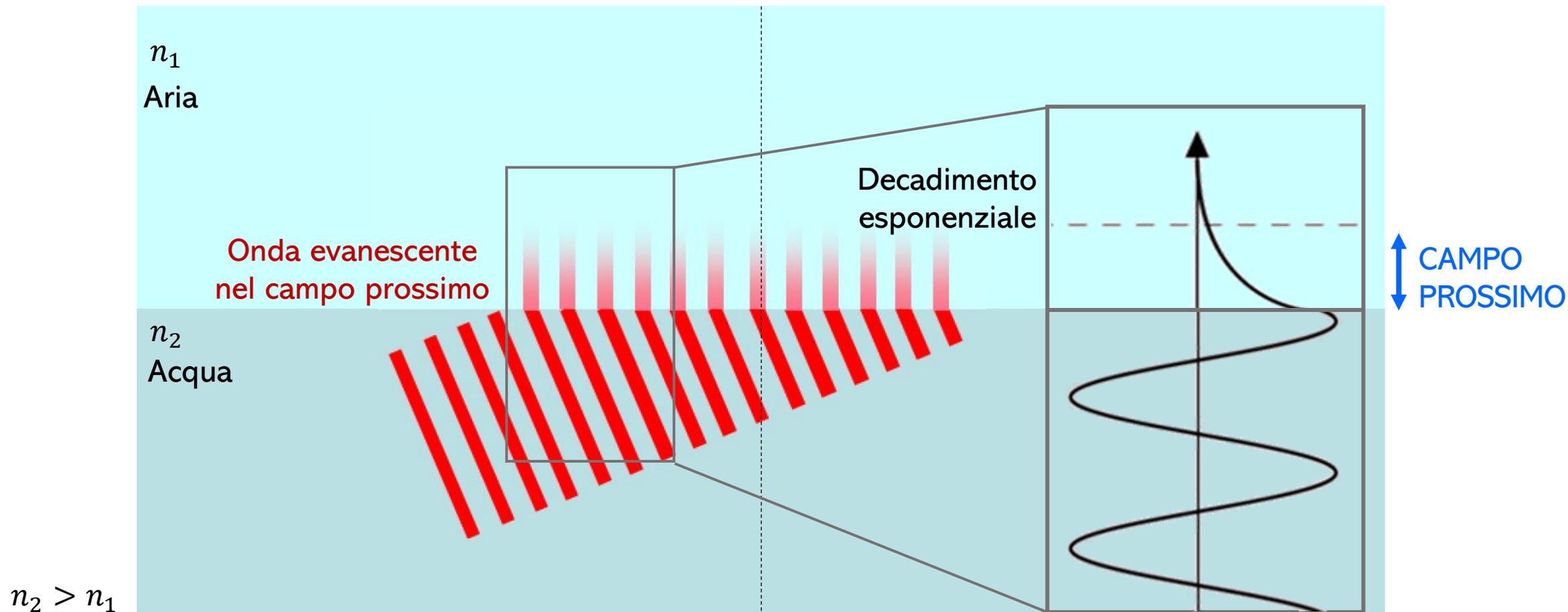




# Riflessione Totale Interna

Legge di Snell

$$n_1 \sin \theta_i = n_2 \sin \theta_t$$

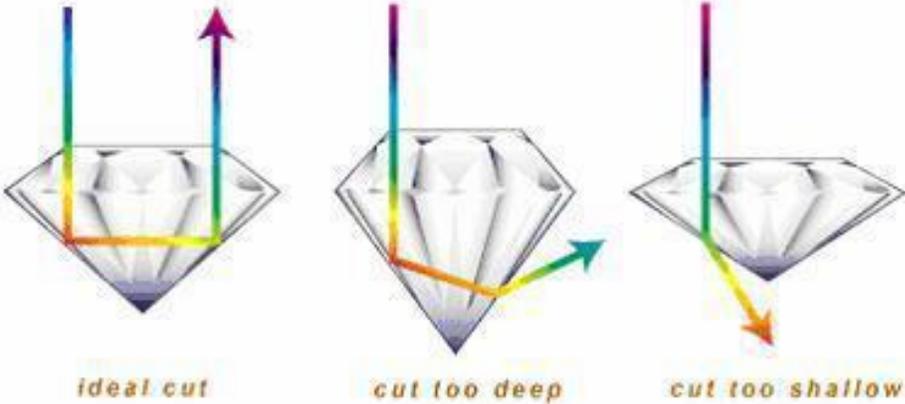


# Riflessione Totale Interna



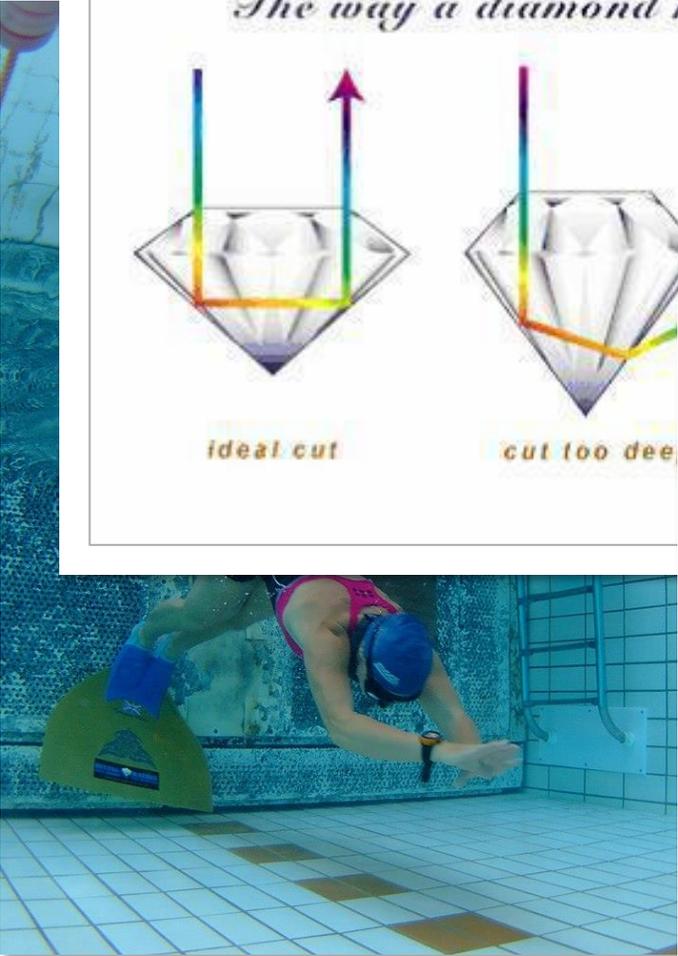
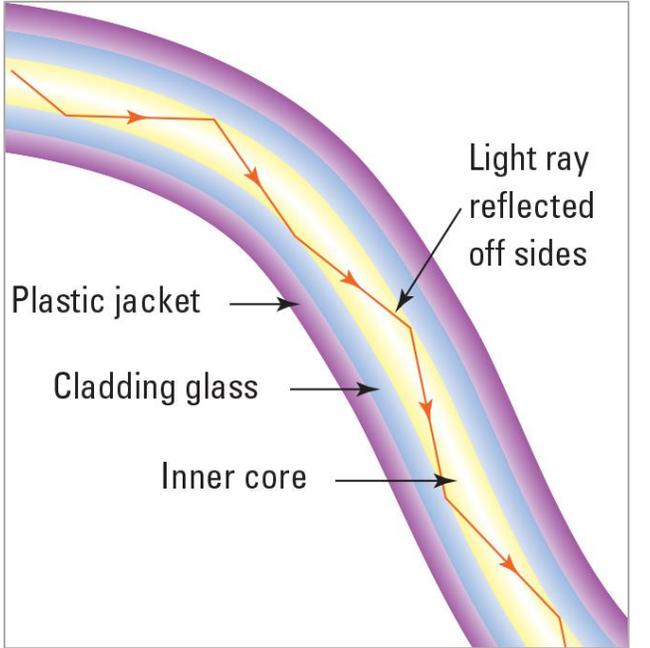
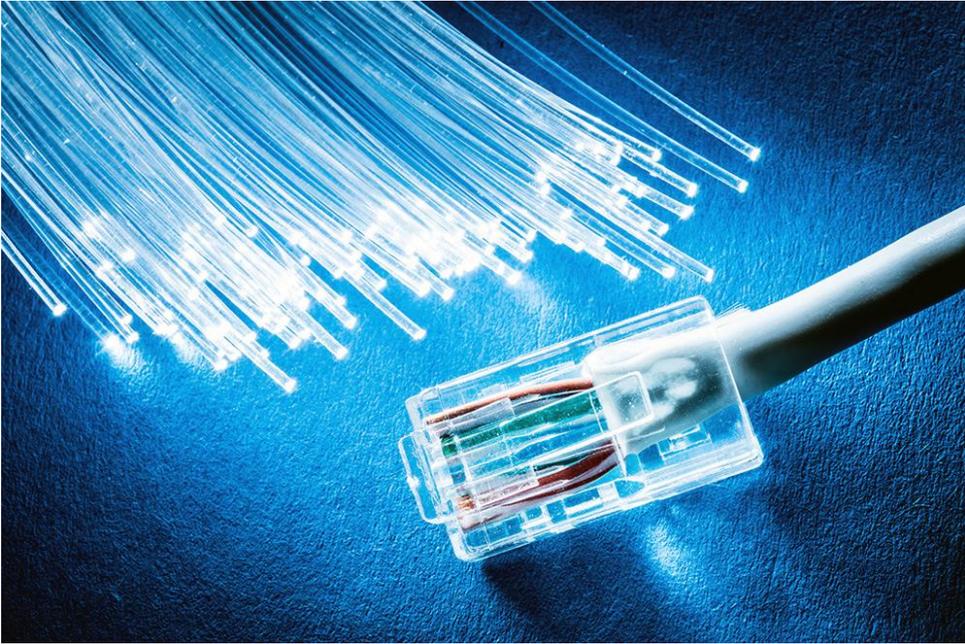
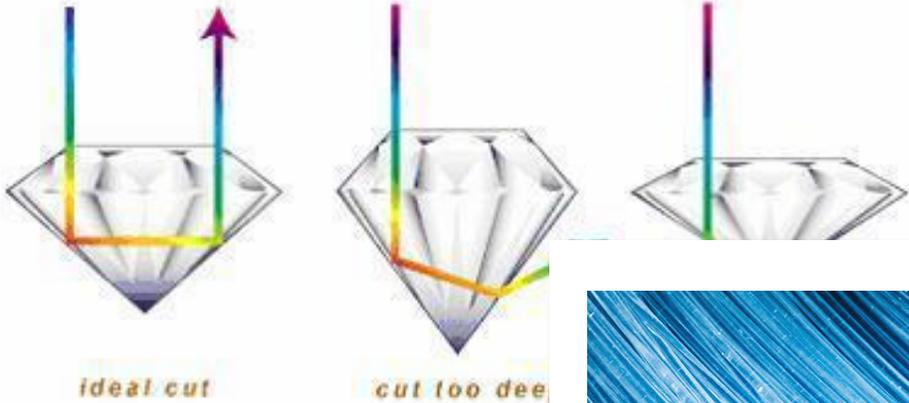
# Riflessione Totale Interna

*The way a diamond handles light*



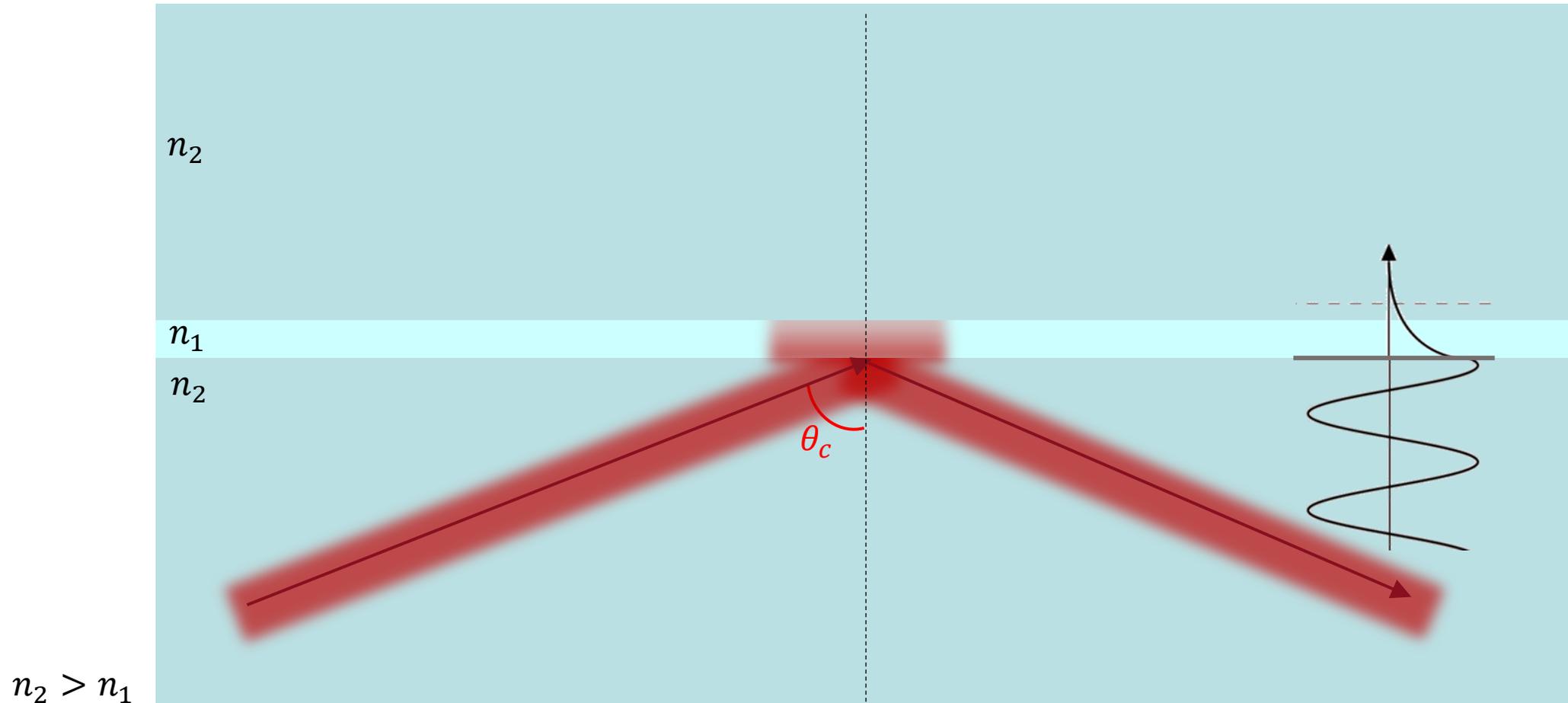
# Riflessione Totale Interna

*The way a diamond handles light*



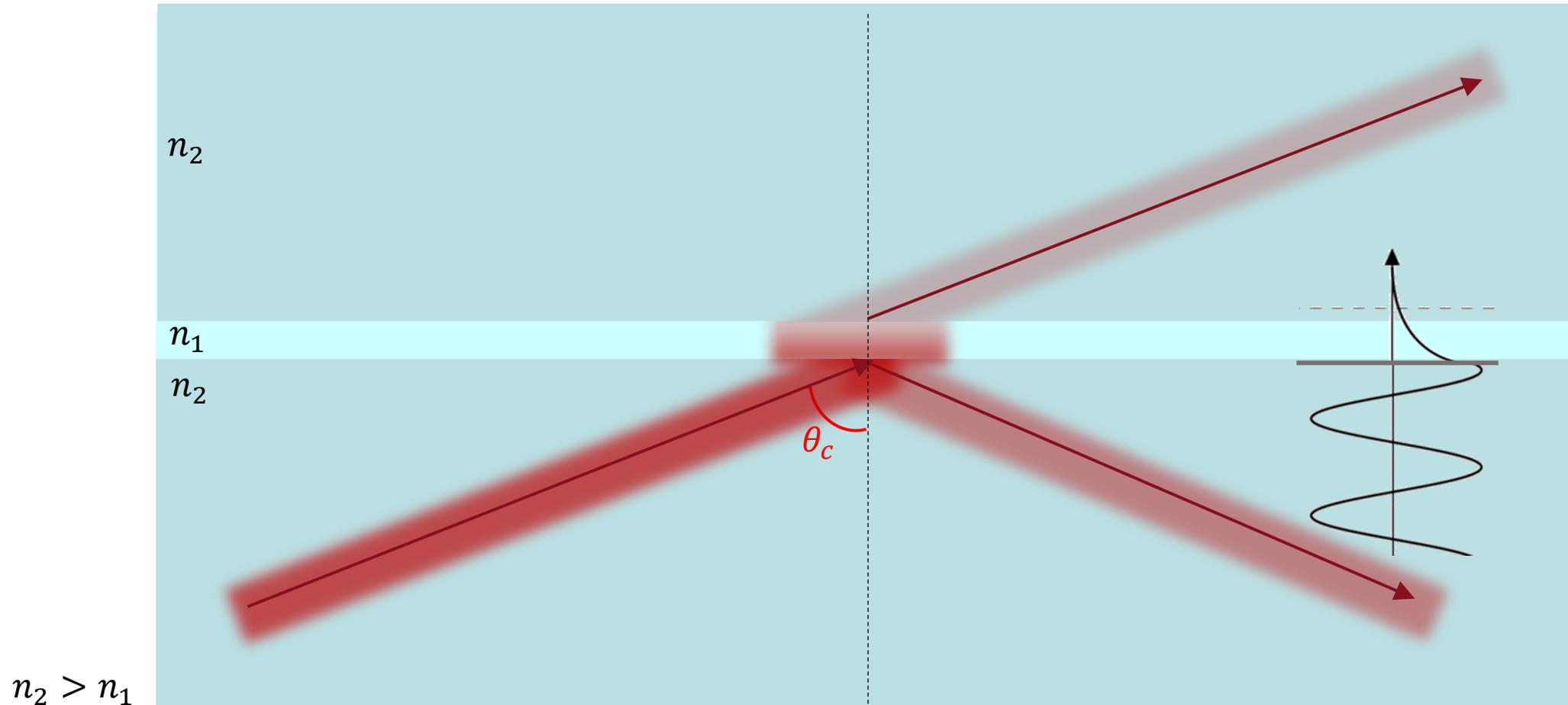
# Riflessione Totale Interna Frustrata (FTIR)

Si può “trasformare” un’onda evanescente in un’onda propagante



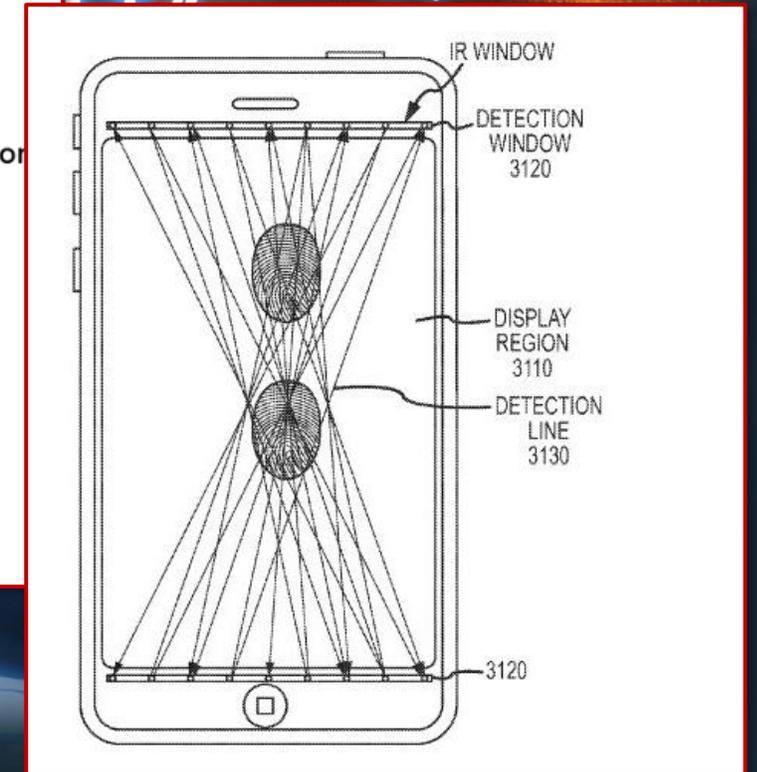
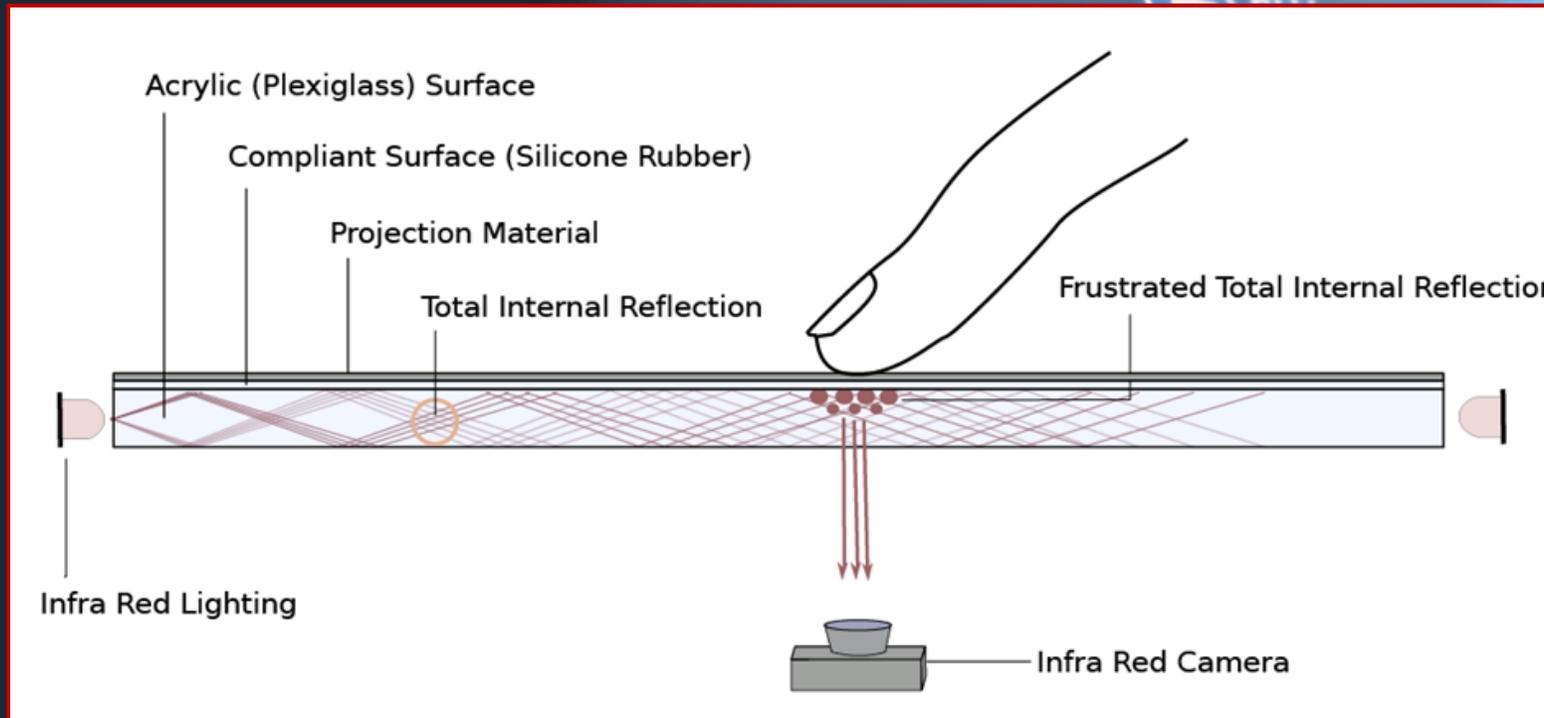
# Riflessione Totale Interna Frustrata (FTIR)

Si può “trasformare” un’onda evanescente in un’onda propagante



# Riflessione Totale Interna Frustrata (FTIR)

## Dispositivi Touch Screen

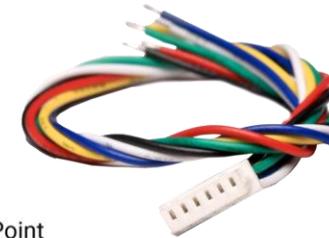
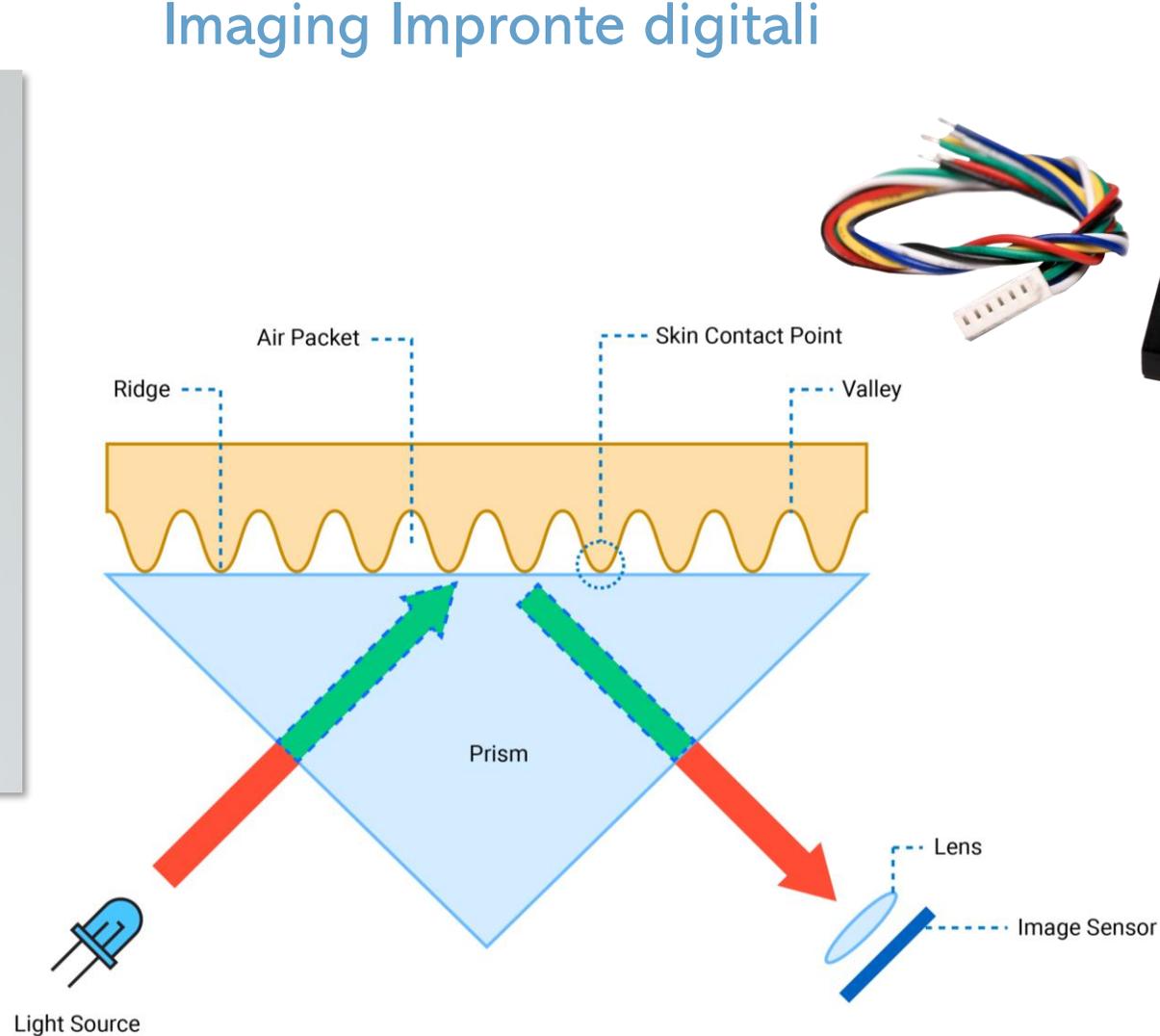


# Riflessione Totale Interna Frustrata (FTIR)

## Imaging Impronte digitali



Impronte digitali evidenziate dentro il bicchiere, a causa di FTIR. Le impronte osservate sono circondate da aree opache dove si ha riflessione totale interna.



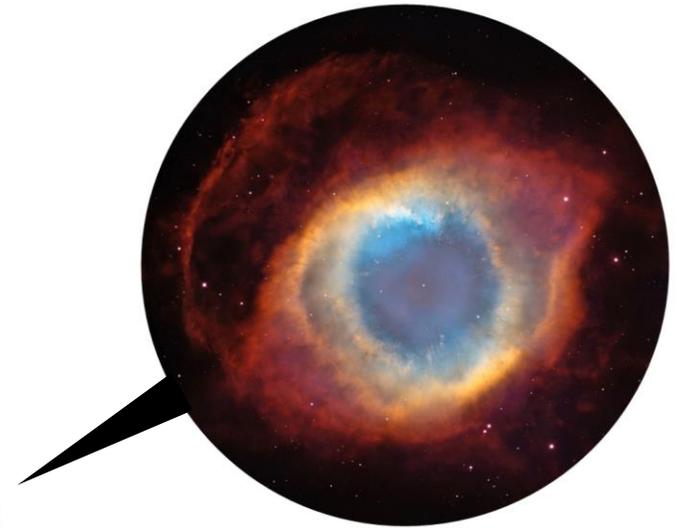
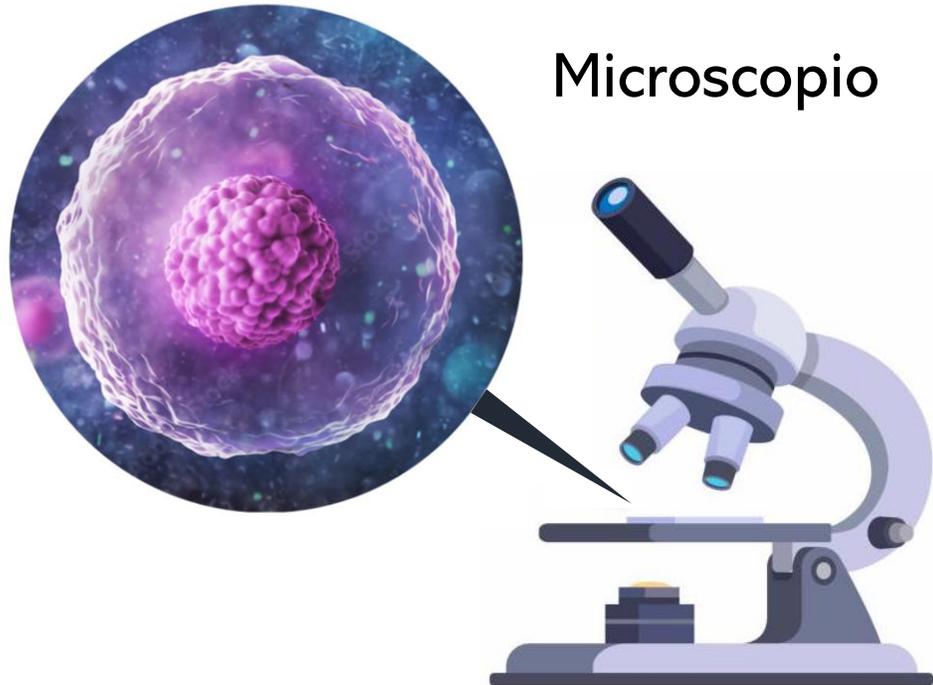
# Risoluzione Ottica



# Risoluzione Ottica

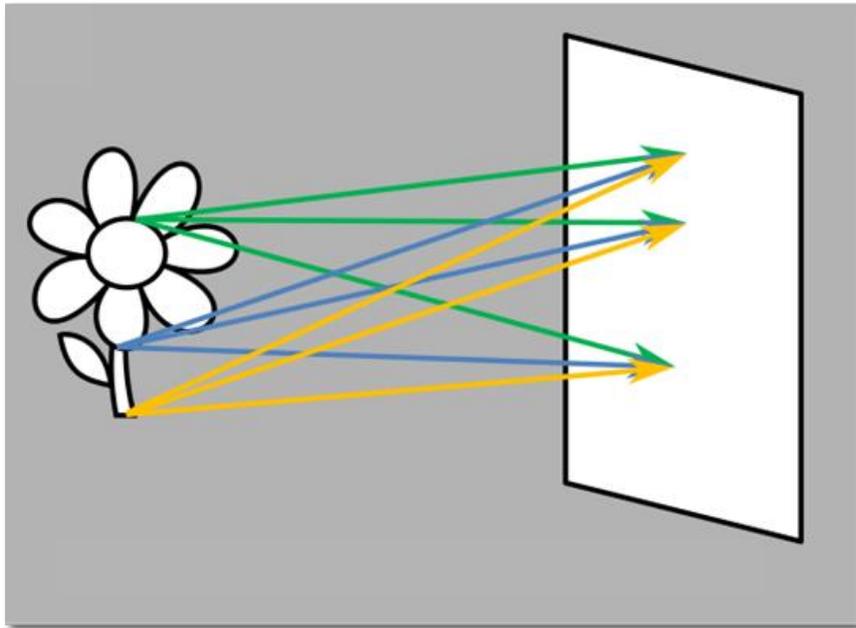
La risoluzione ottica è una proprietà degli strumenti ottici che ci permettono di visualizzare, di fare immagini del mondo circostante.

E' la capacità di distinguere dettagli e particolari che sono separati nel campione in osservazione anche nell'immagine del campione fornita dallo strumento ottico.



# Risoluzione Ottica

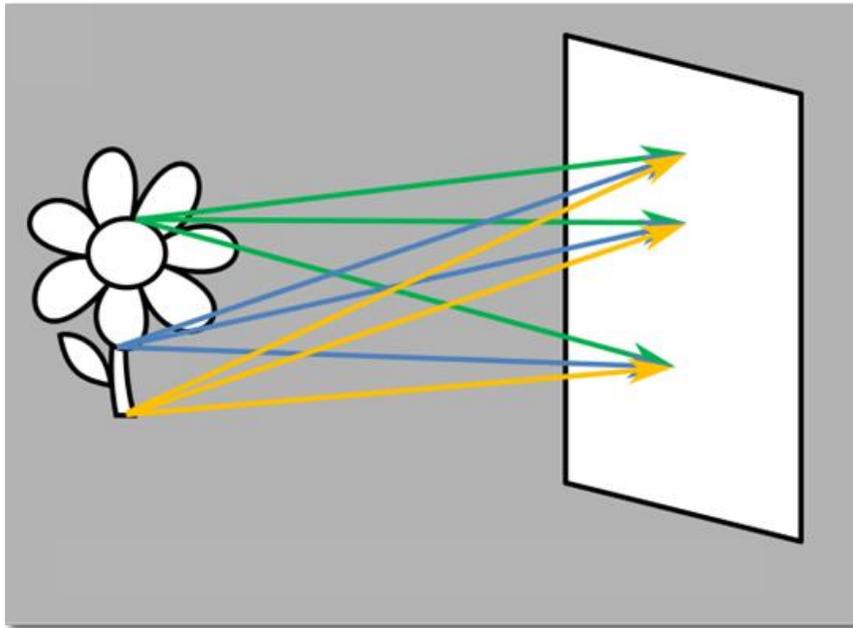
La formazione dell'immagine



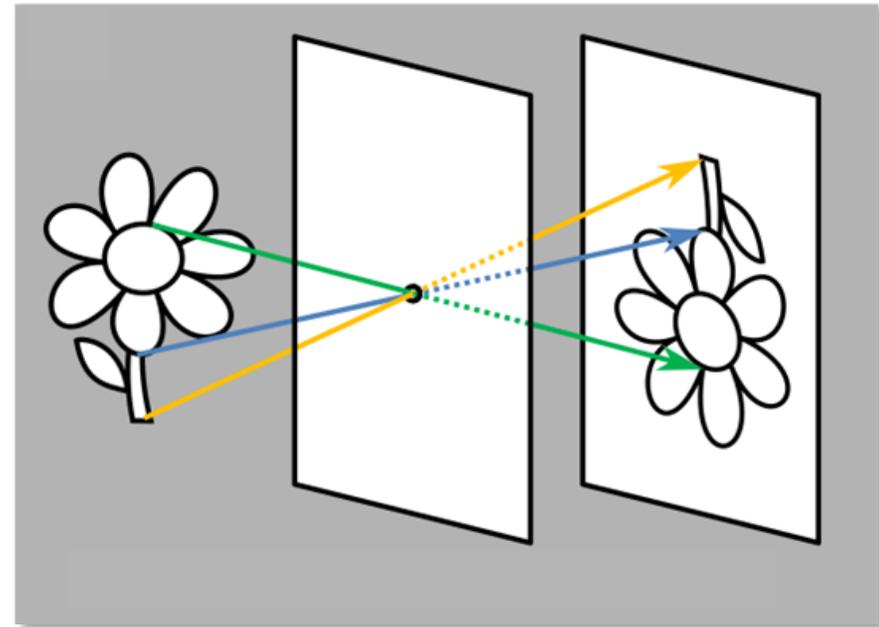
Raggi della luce provenienti da diversi punti dell'oggetto si sovrappongono sullo schermo.

# Risoluzione Ottica

## La formazione dell'immagine



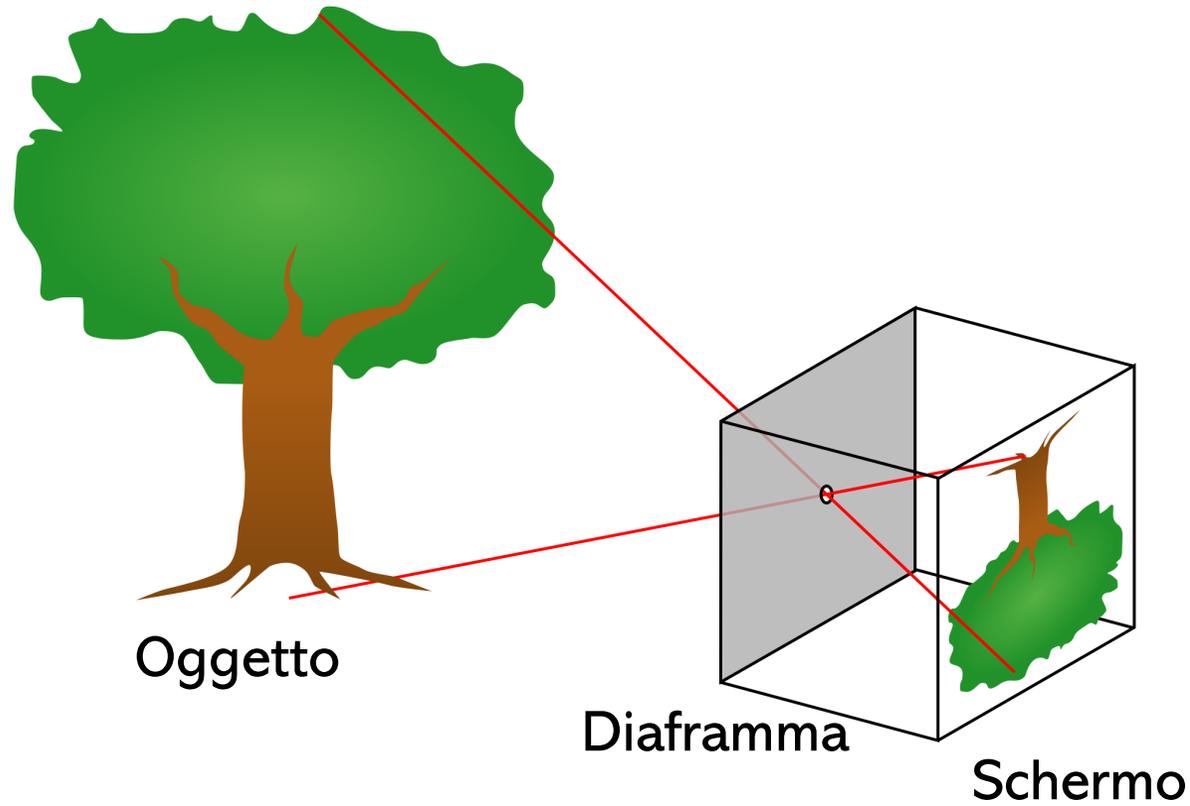
Raggi della luce provenienti da diversi punti dell'oggetto si sovrappongono sullo schermo.



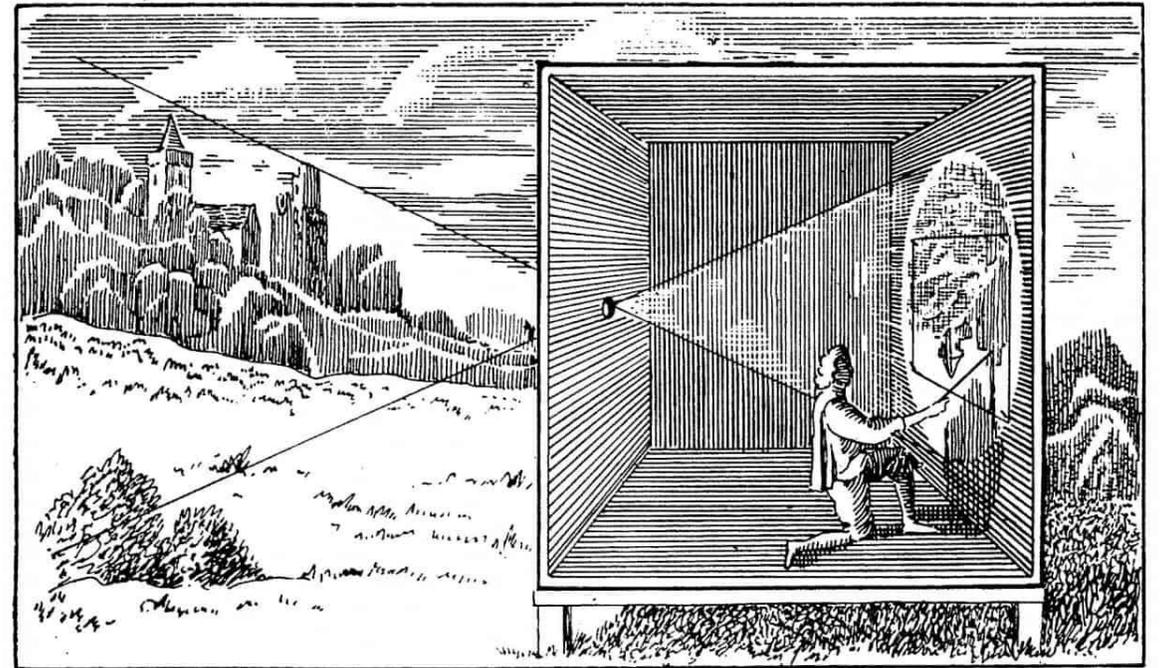
Un diaframma filtra i raggi, determinando una corrispondenza uno-a-uno tra i punti dell'oggetto e i punti sullo schermo.

# Risoluzione Ottica

La formazione dell'immagine

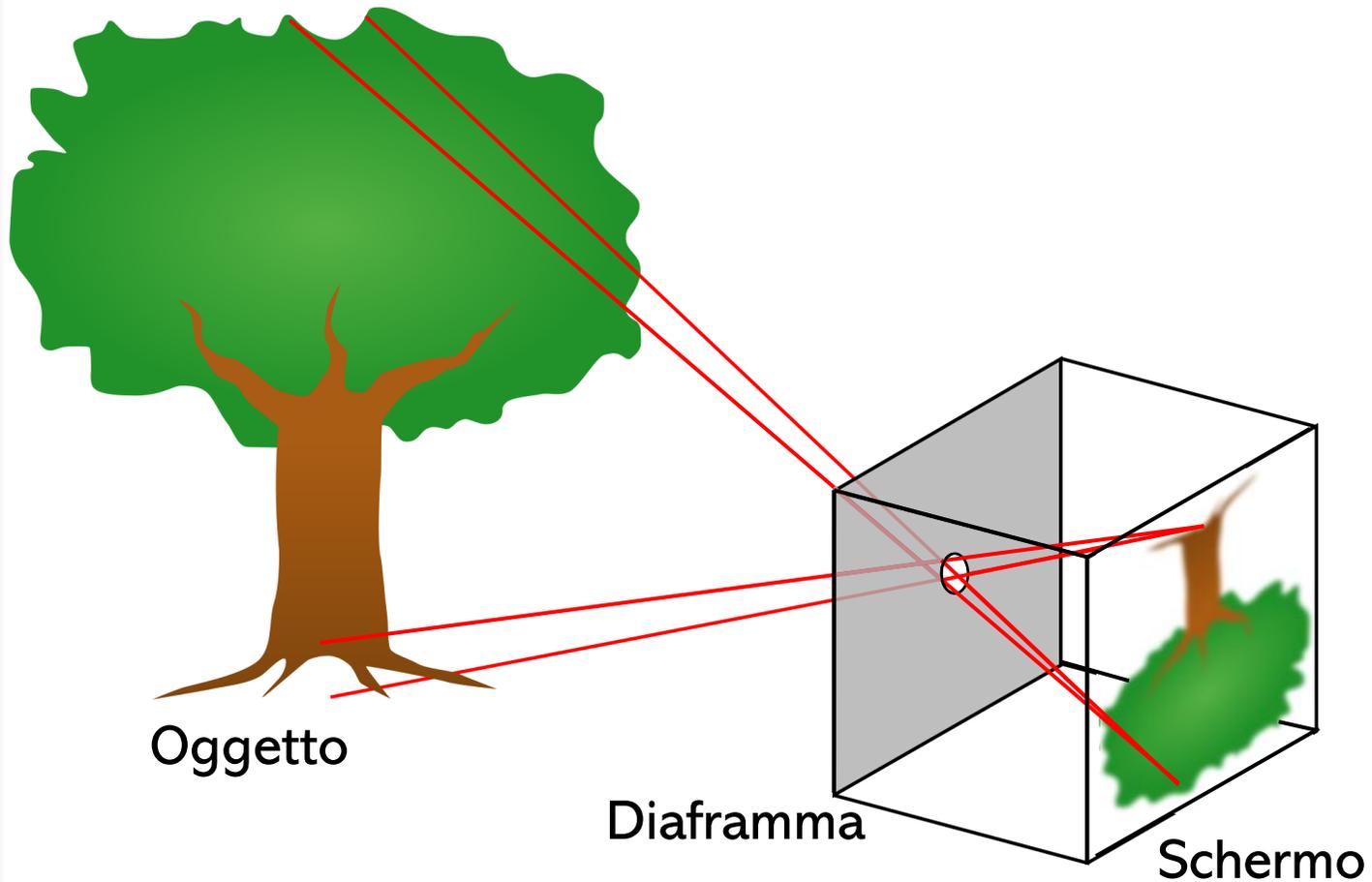


→ Principio della *Camera Oscura*



# Risoluzione Ottica

La formazione dell'immagine



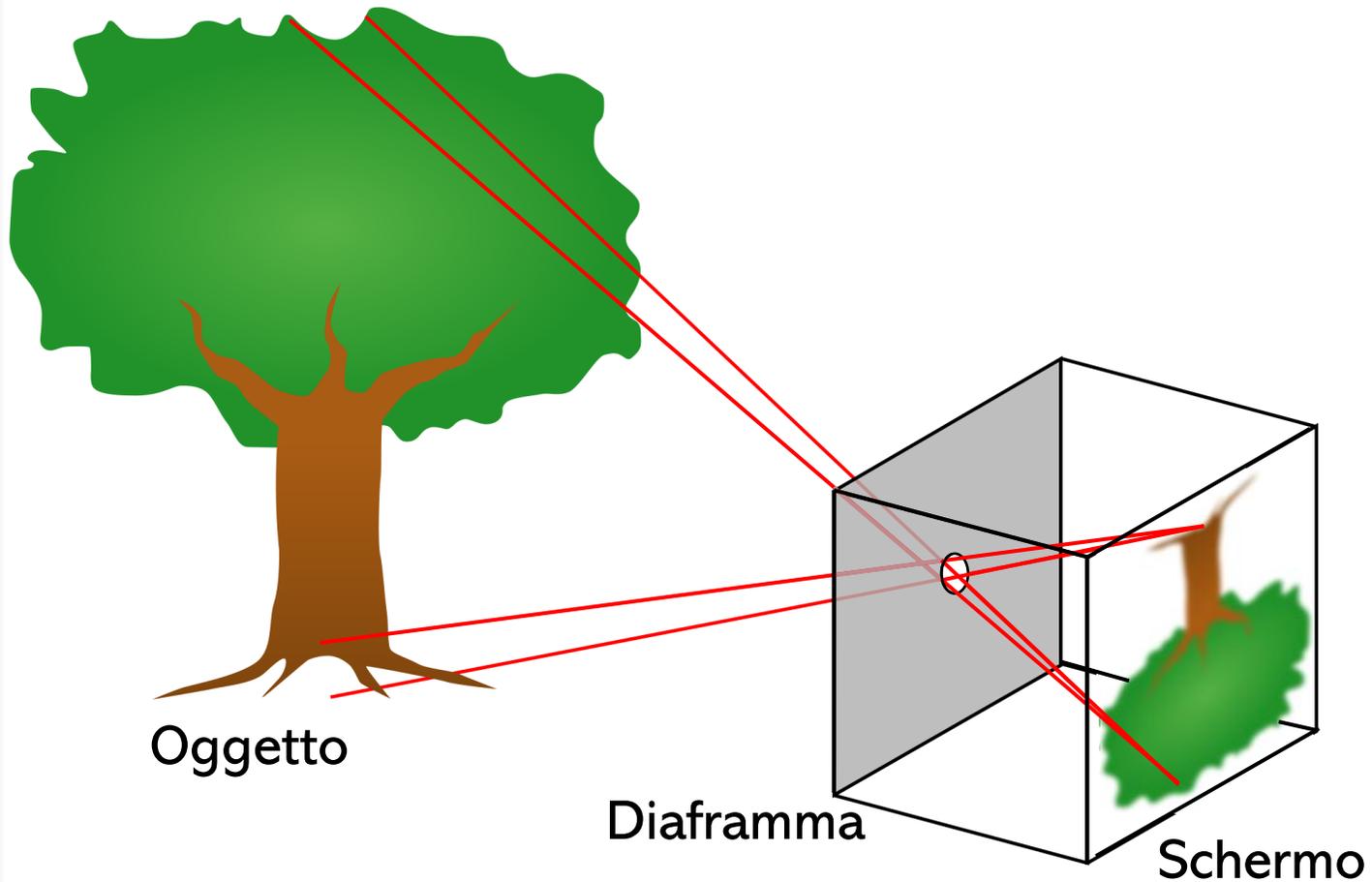
Foro troppo grande:  
Immagine sfocata



Scarso filtro spaziale

# Risoluzione Ottica

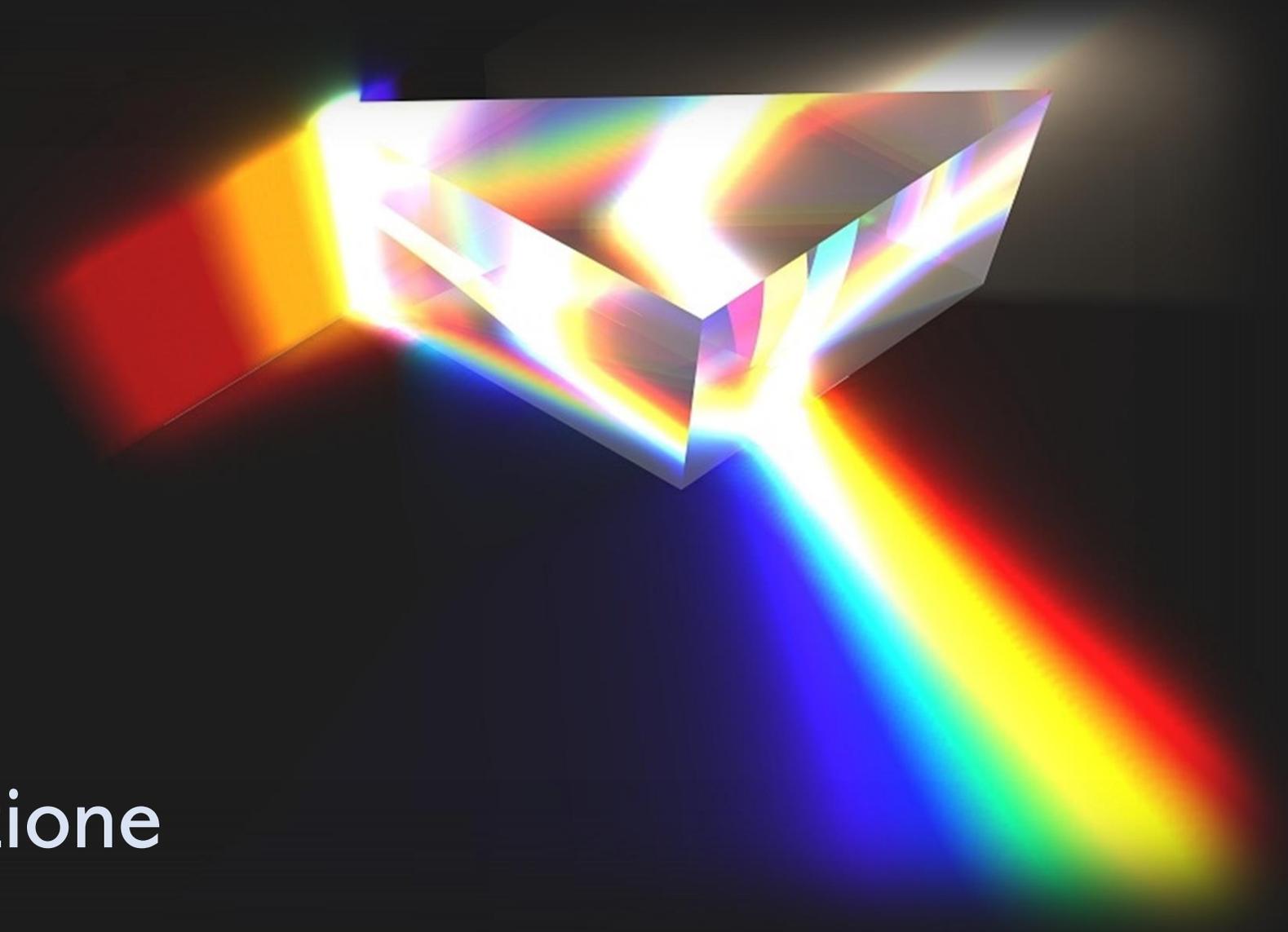
La formazione dell'immagine



Foro troppo piccolo:  
Immagine sfocata

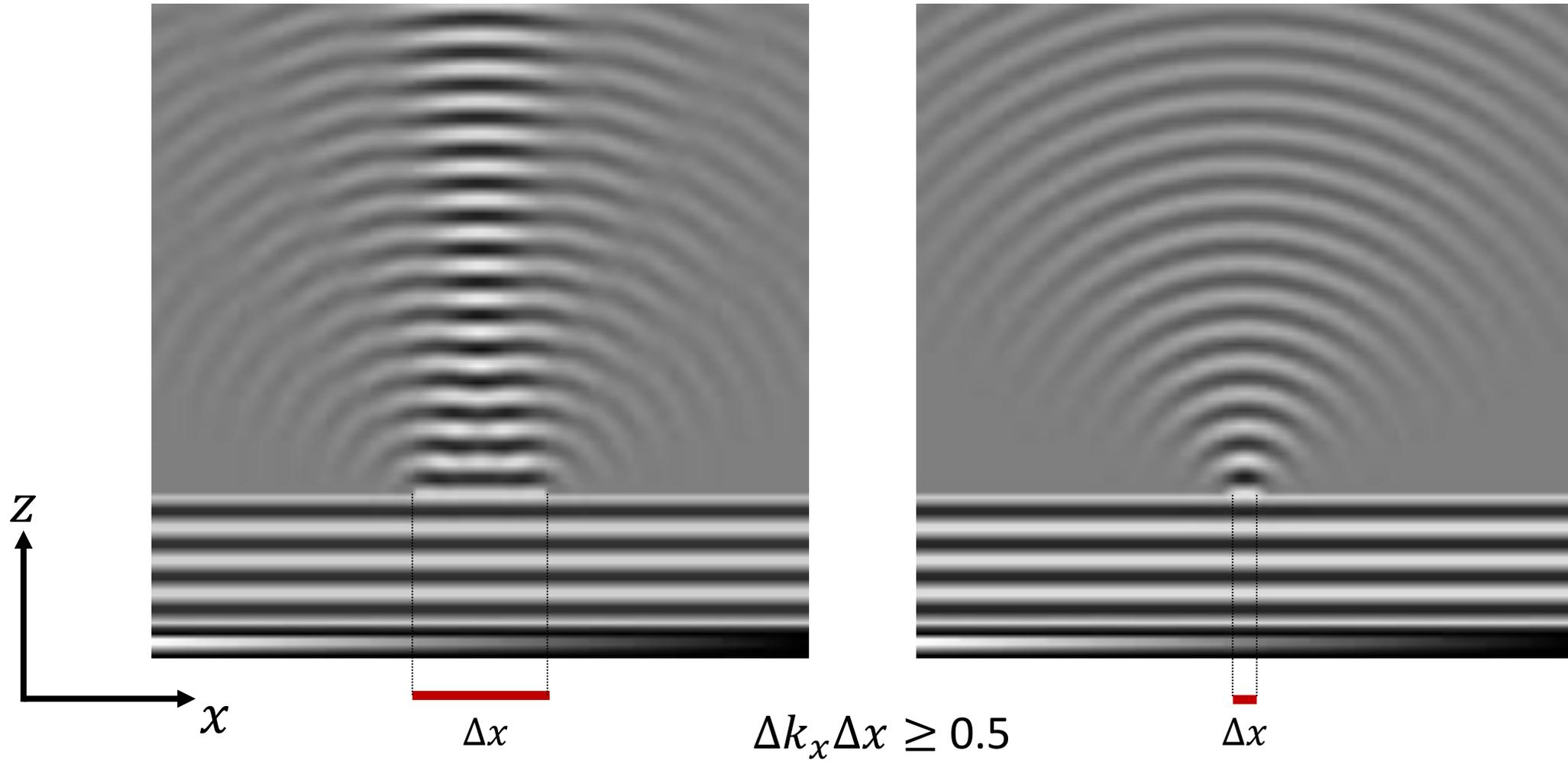


**DIFFRAZIONE**

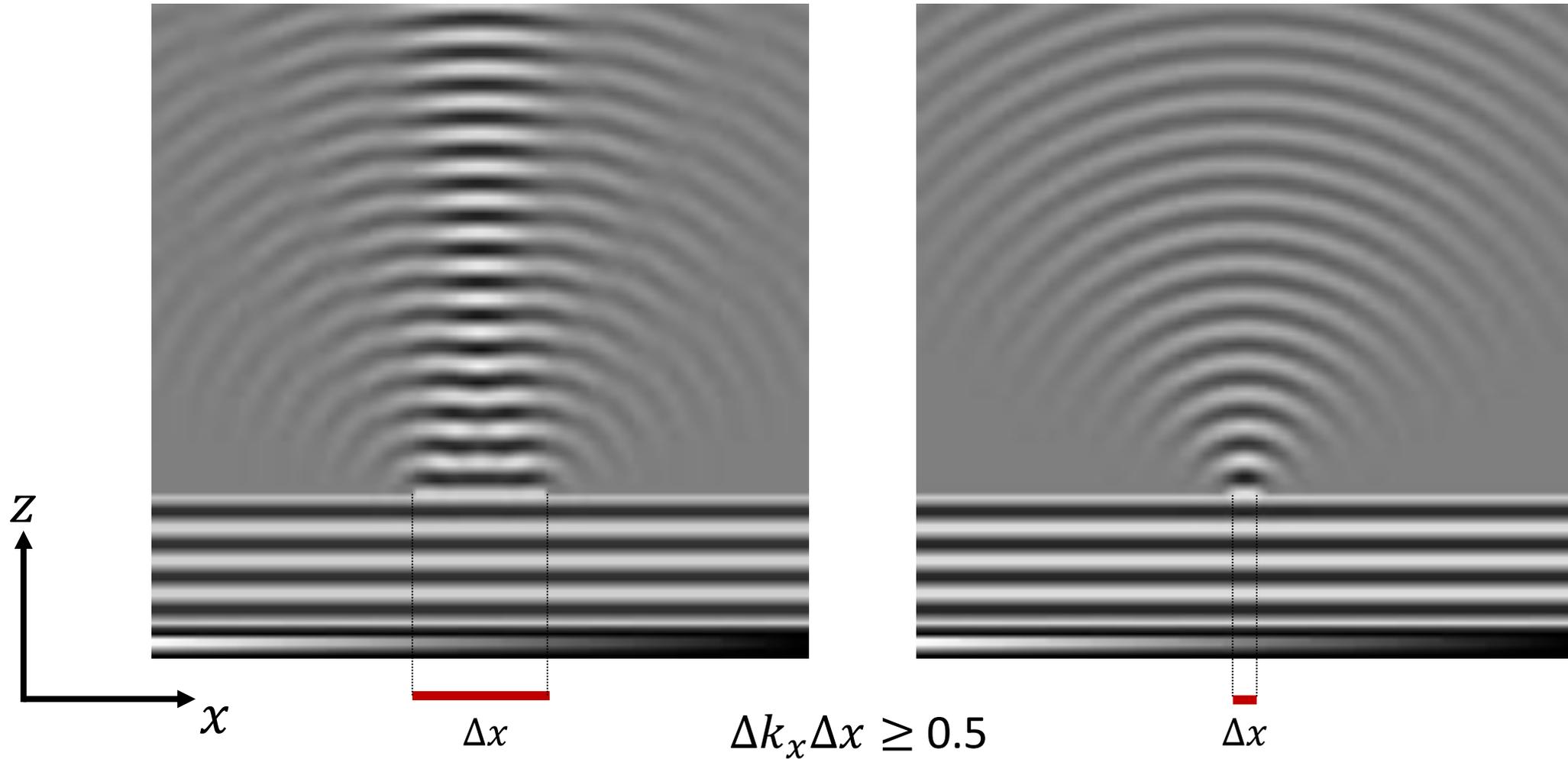


Diffrazione

# Diffrazione



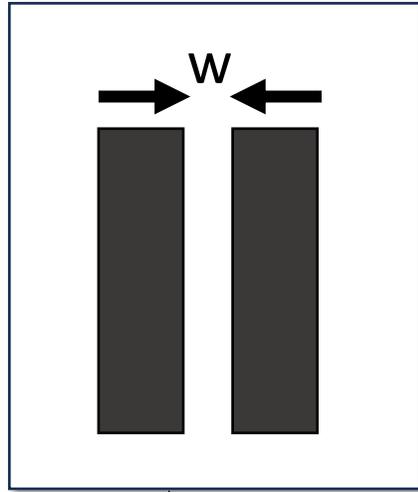
# Diffrazione



Più la dimensione della fenditura è piccola rispetto a  $\lambda$ , maggiori saranno le componenti  $\Delta k_x$

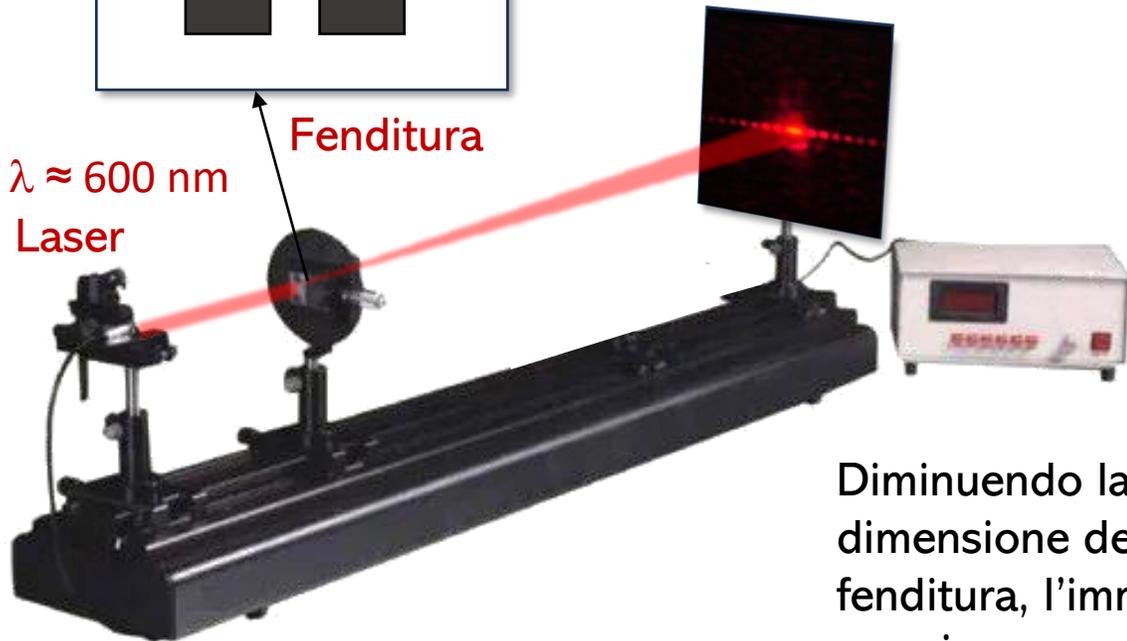
# Diffrazione

## Laser attraverso una fenditura

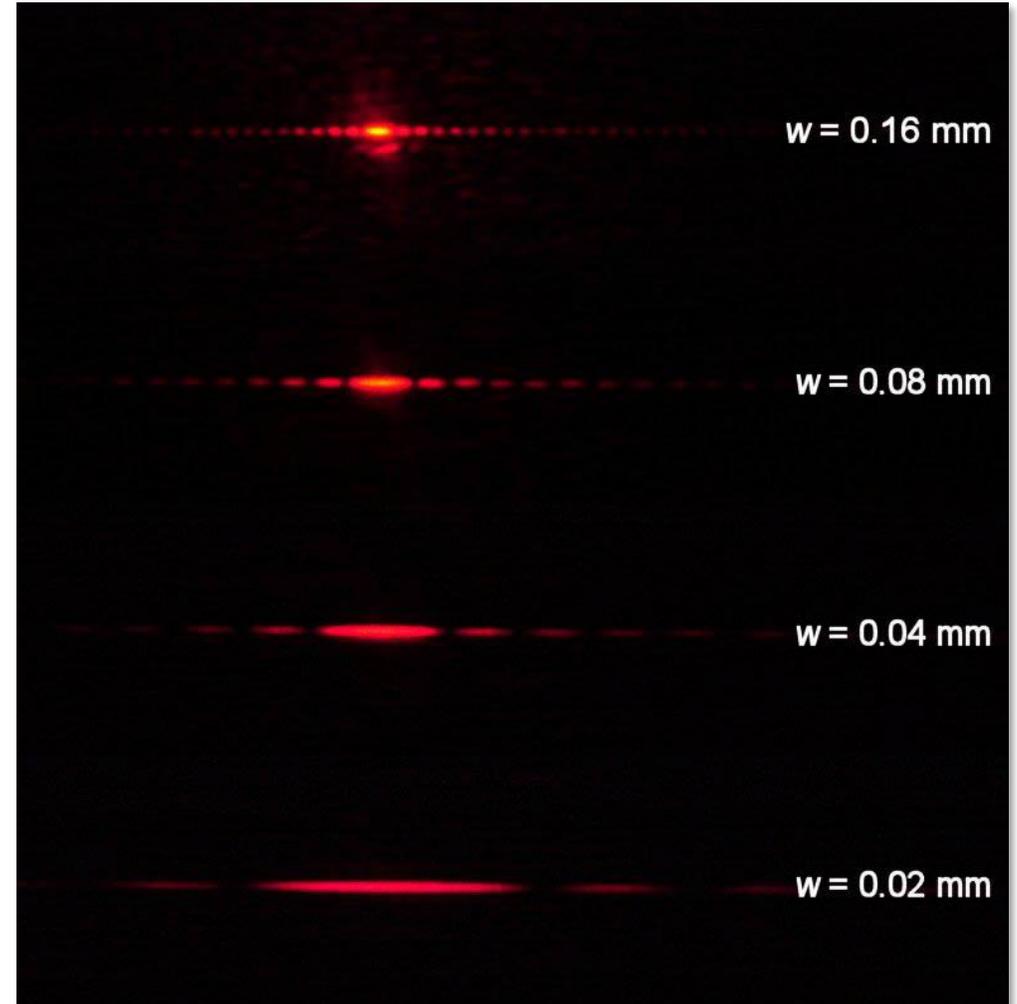


$\lambda \approx 600 \text{ nm}$   
Laser

Fenditura



Diminuendo la  
dimensione della  
fenditura, l'immagine  
peggiora

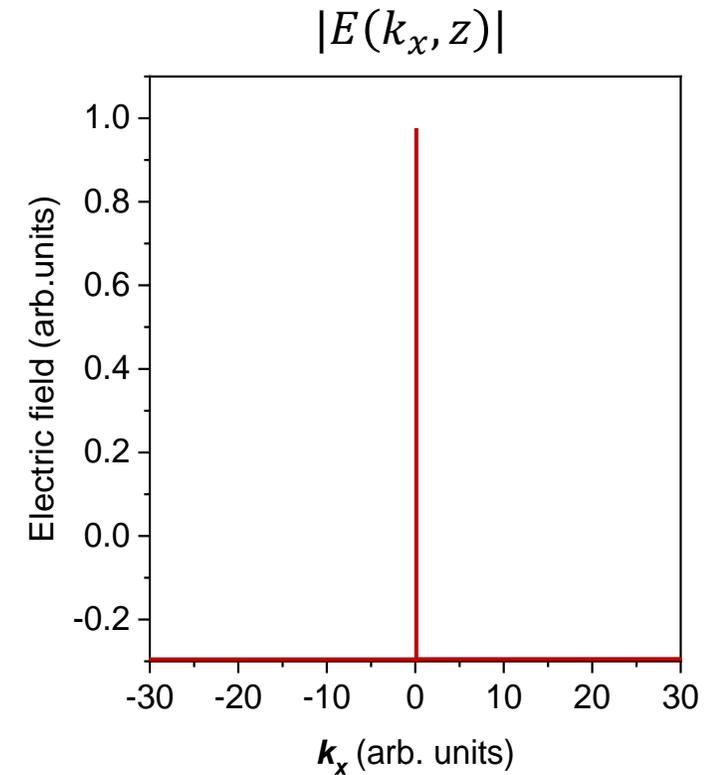
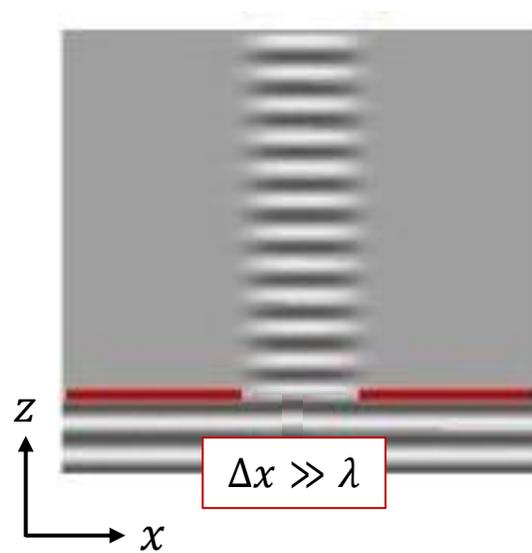
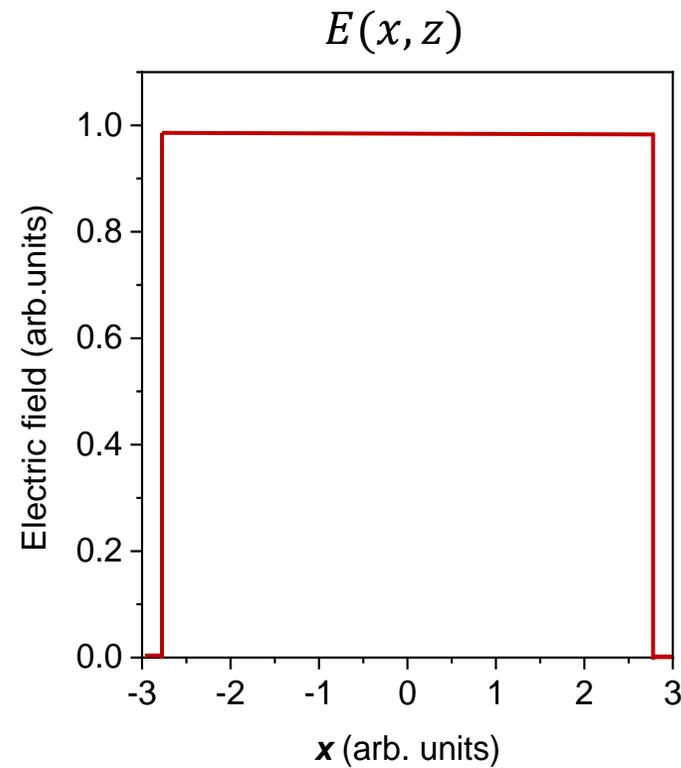


# Diffrazione e Risoluzione Ottica

Sorgente locale confinata in  $x$  (piano oggetto)

Trasformata di Fourier  
← Propagazione lungo  $z$  →

Campo elettrico dopo la propagazione  
(di cui fare imaging)

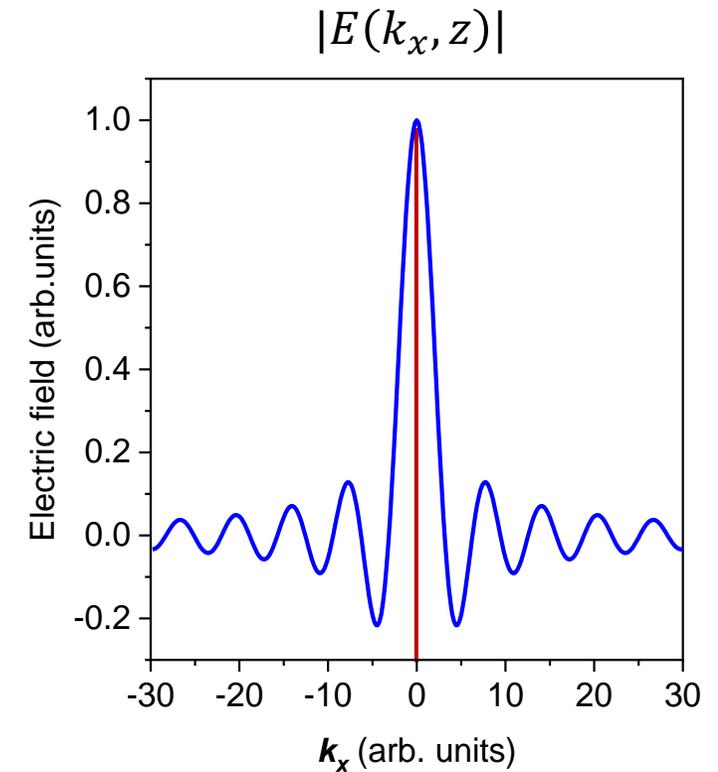
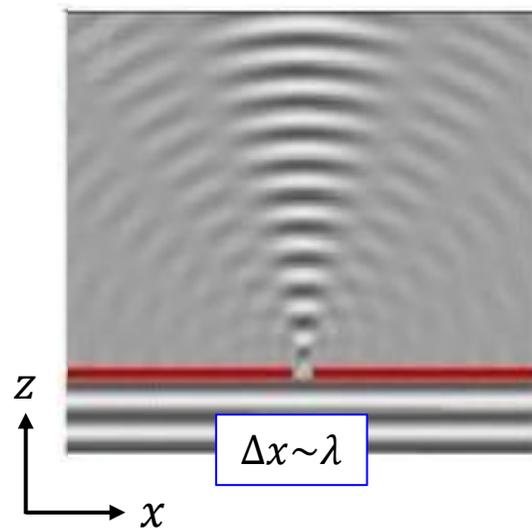
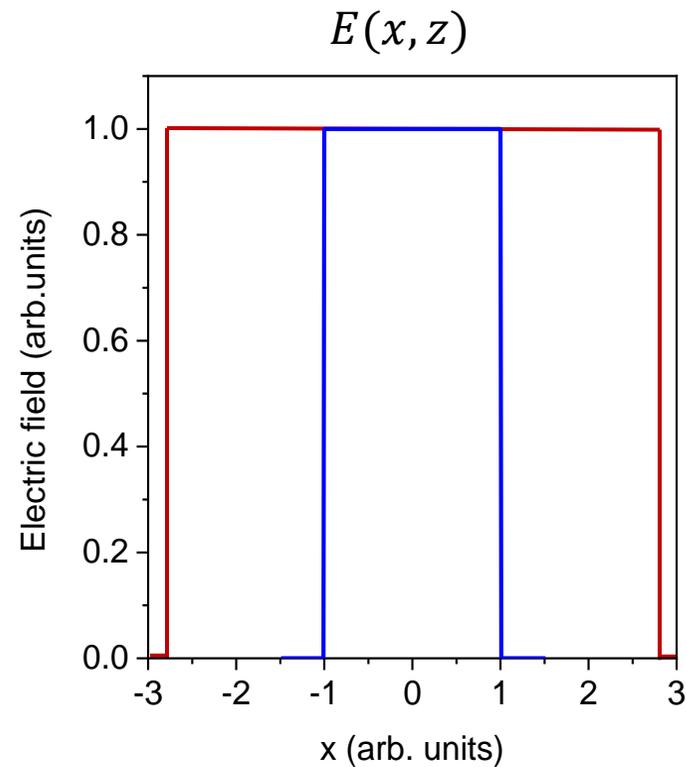


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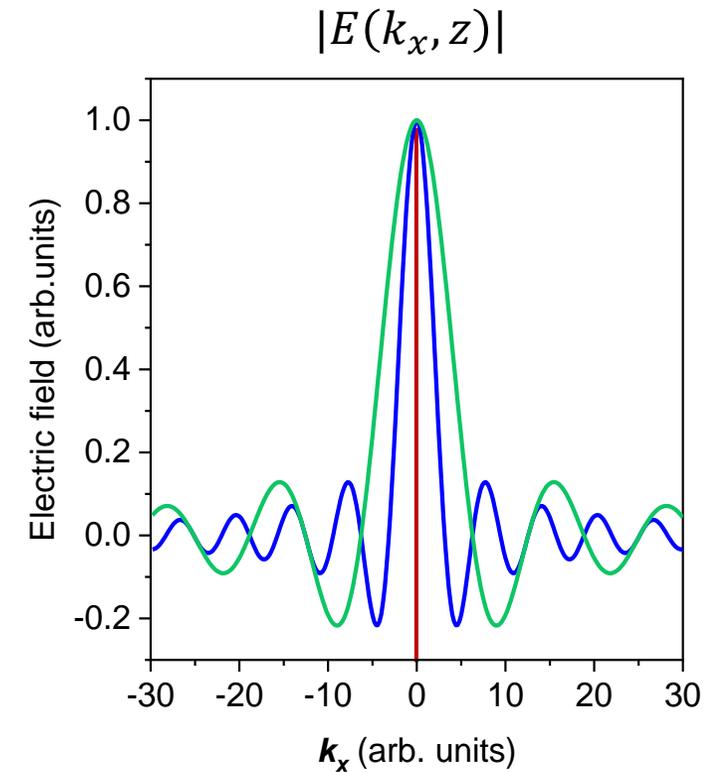
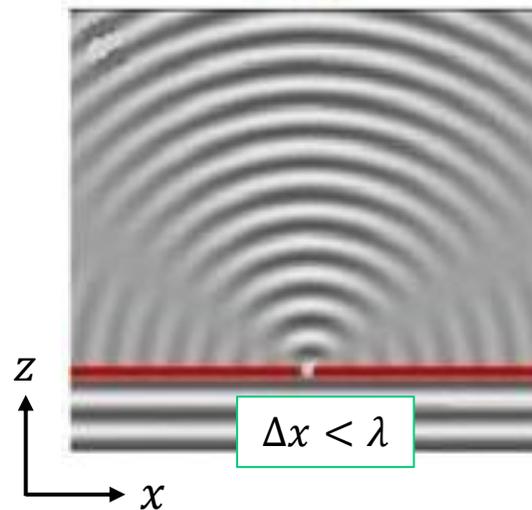
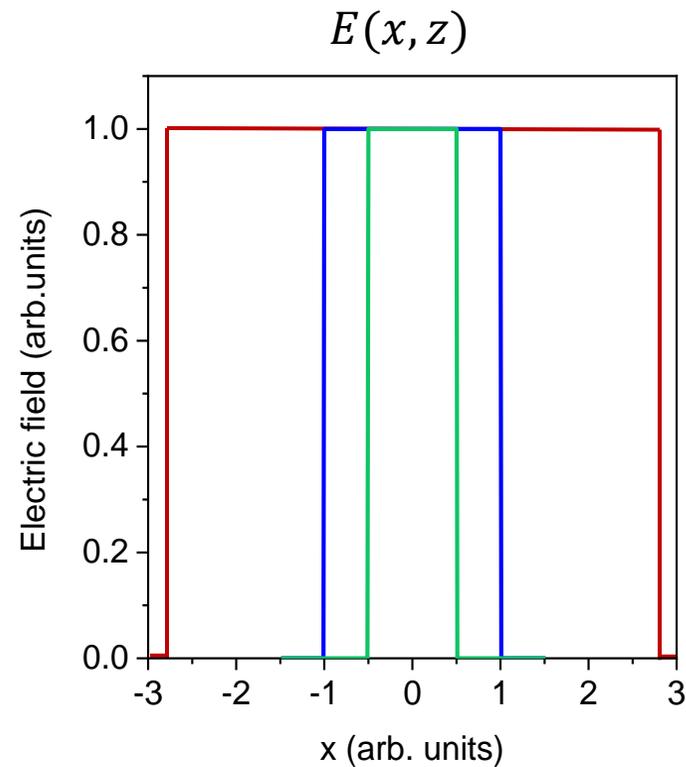


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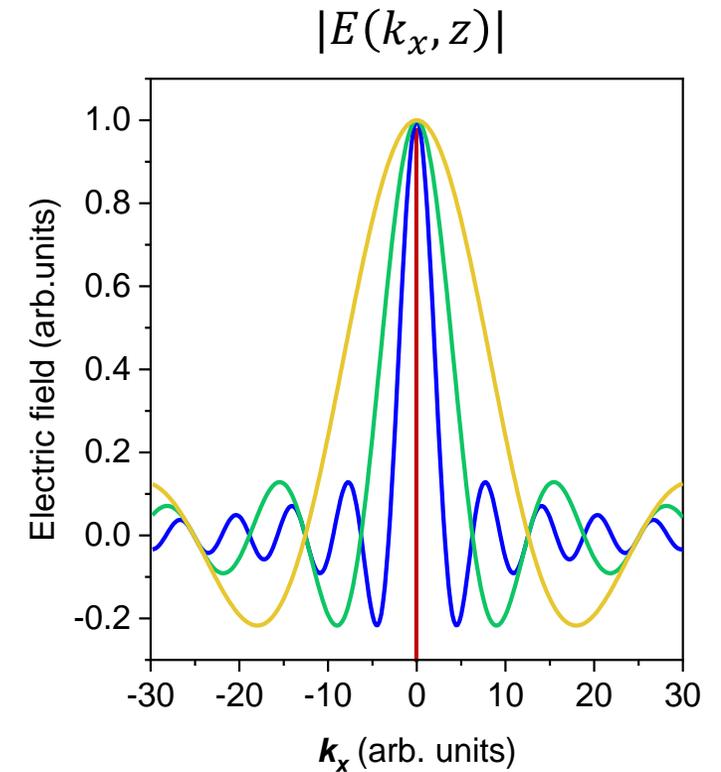
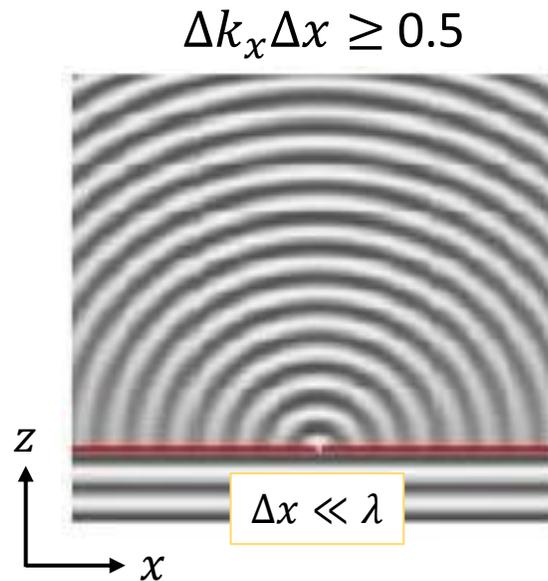
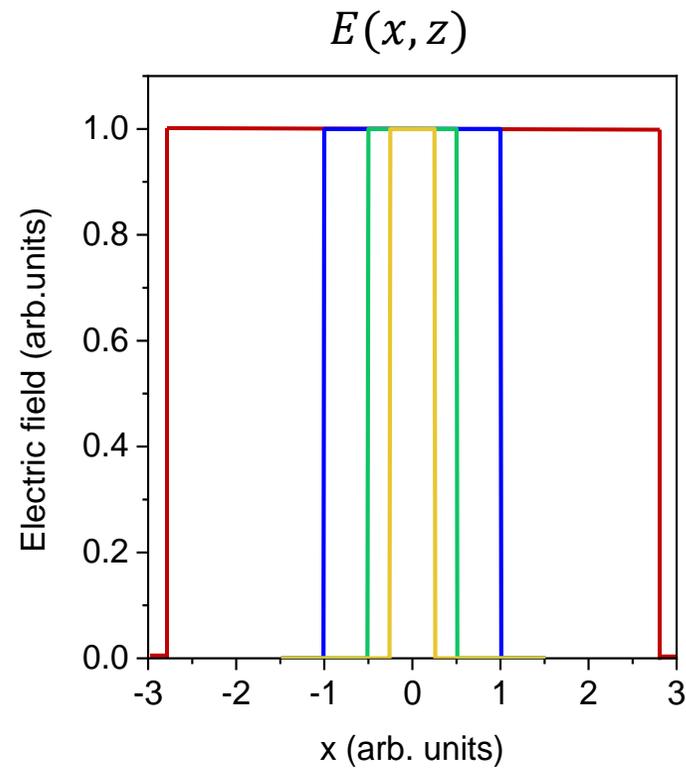


# Diffrazione e Risoluzione Ottica

Sorgente locale confinata in  $x$  (piano oggetto)

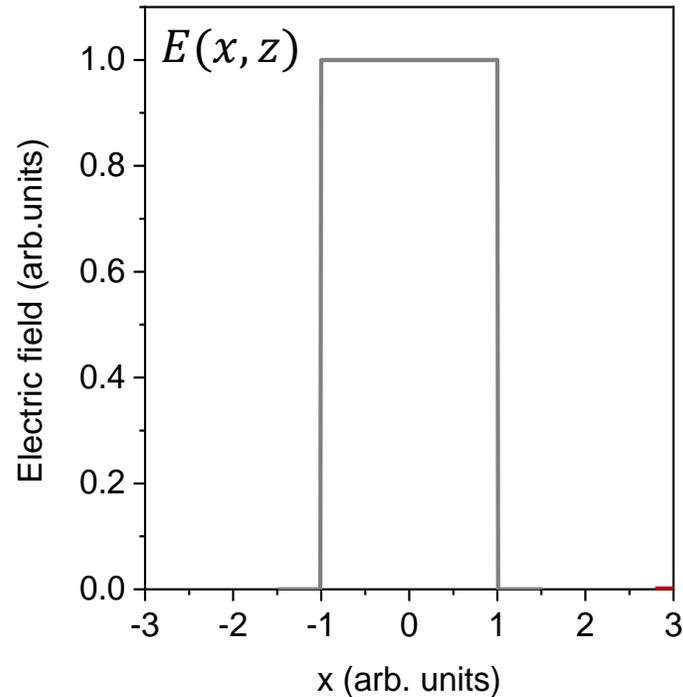
Trasformata di Fourier  
← Propagazione lungo  $z$  →

Campo elettrico dopo la propagazione  
(di cui fare imaging)



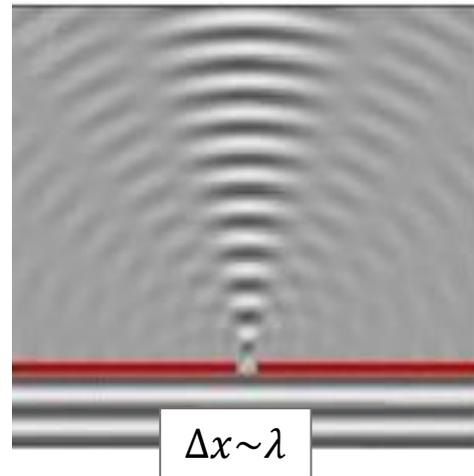
# Diffrazione e Risoluzione Ottica

Sorgente locale confinata in x (piano oggetto)

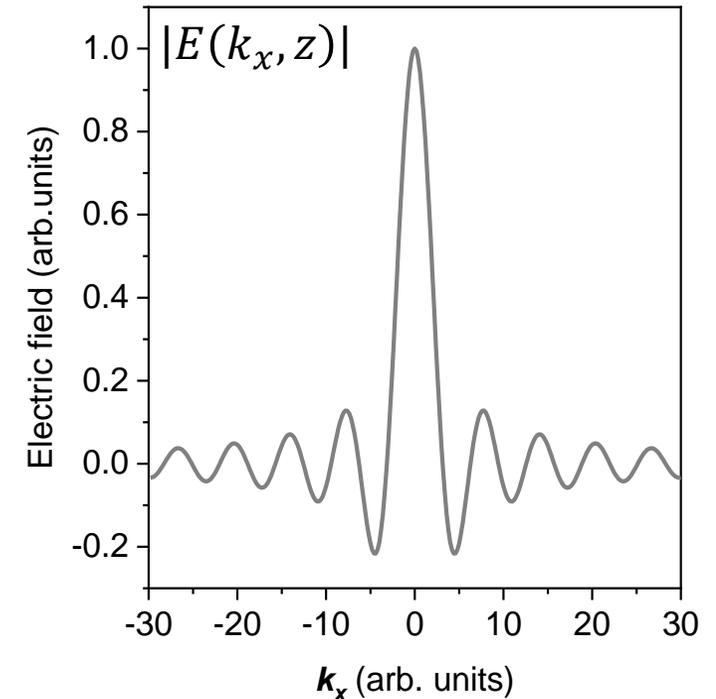


Trasformata di Fourier  
← Propagazione lungo z →

$$\Delta k_x \Delta x \geq 0.5$$



Campo elettrico dopo la propagazione  
(di cui fare imaging)

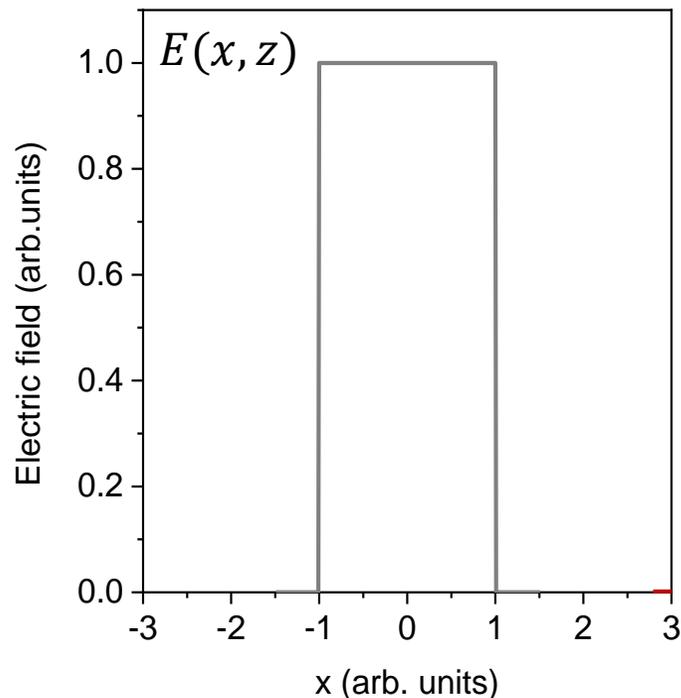


La luce acquista  $k$  laterali perché sto cercando di confinarla, ma  $k_x$  non può superare il suo modulo!

$$k = \frac{\omega}{c} = \frac{2\pi}{\lambda}$$

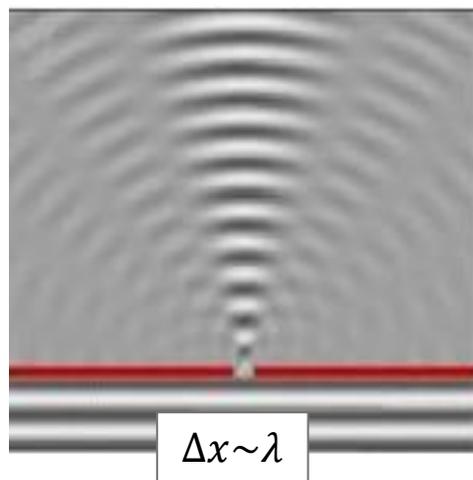
# Diffrazione e Risoluzione Ottica

Sorgente locale confinata in x (piano oggetto)

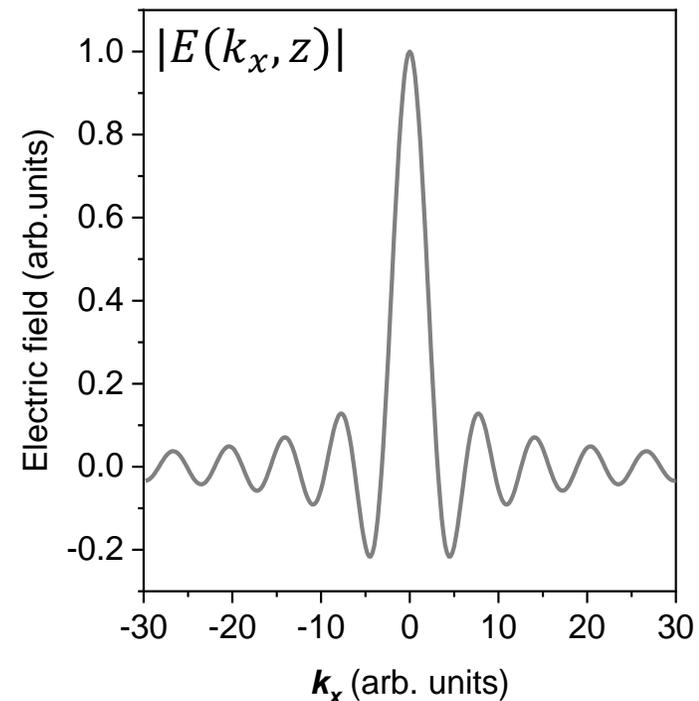


Trasformata di Fourier  
 $\longleftrightarrow$   
 Propagazione lungo z

$$\Delta k_x \Delta x \geq 0.5$$



Campo elettrico dopo la propagazione  
 (di cui fare imaging)



La luce acquista  $k$  laterali perché sto cercando di confinarla, ma  $k_x$  non può superare il suo modulo!

$$k = \frac{\omega}{c} = \frac{2\pi}{\lambda}$$

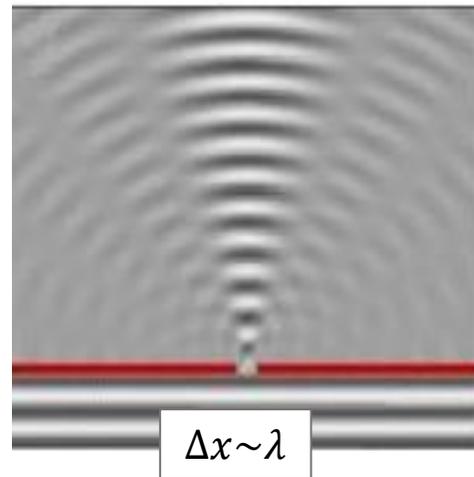
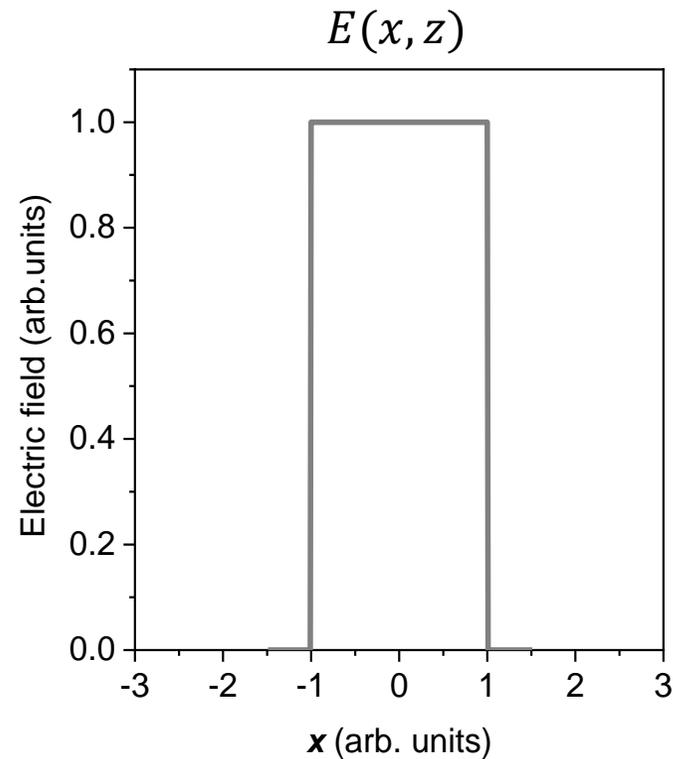
Perciò nella trasformata di Fourier non posso integrare su tutti i  $k$  ma solo su quelli minori e uguali del modulo del momento della luce

# Diffrazione e Risoluzione Ottica

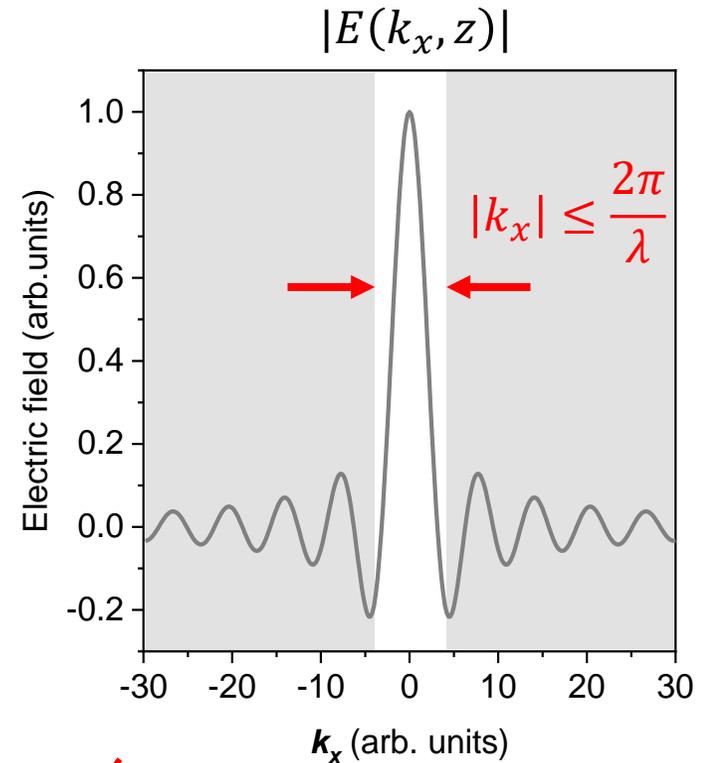
Sorgente locale confinata in  $x$  (piano oggetto)

Trasformata di Fourier  
Propagazione lungo  $z$

Campo elettrico dopo la propagazione  
(di cui fare imaging)



$$\Delta k_x \Delta x \geq 0.5$$



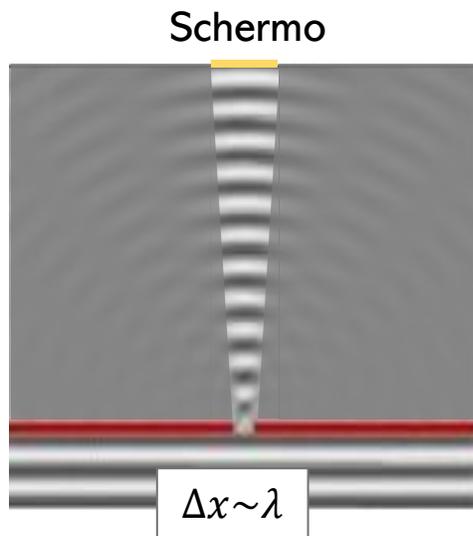
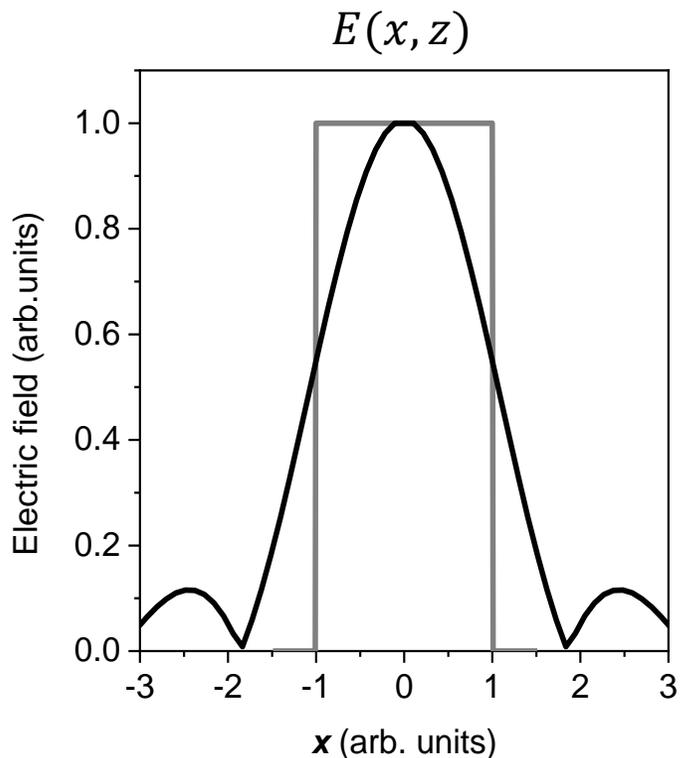
Rifacendo la trasformata applicando questo «filtro» ottengo la curva nera

# Diffrazione e Risoluzione Ottica

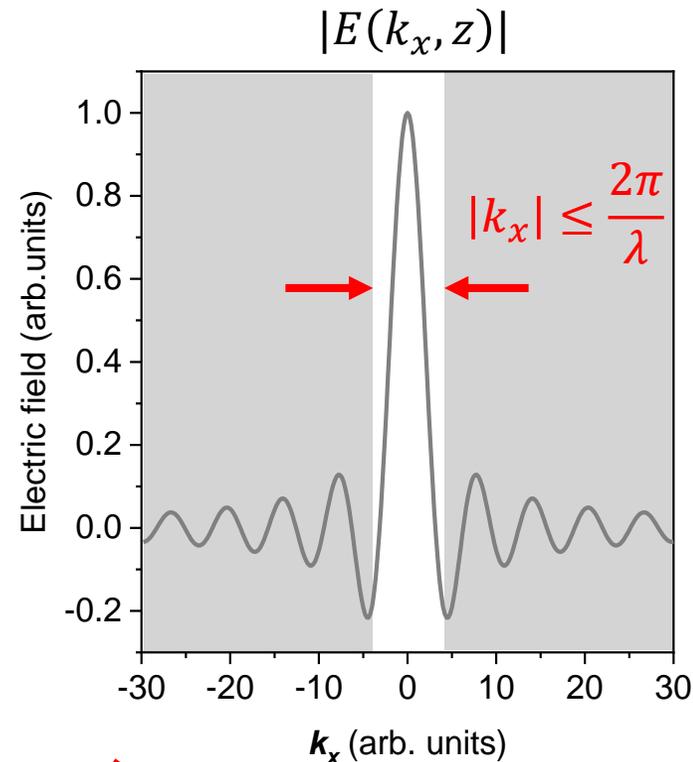
Sorgente locale confinata in  $x$  (piano oggetto)

Trasformata di Fourier  
Propagazione lungo  $z$

Campo elettrico dopo la propagazione  
(di cui fare imaging)



$$\Delta k_x \Delta x \geq 0.5$$



Immagine



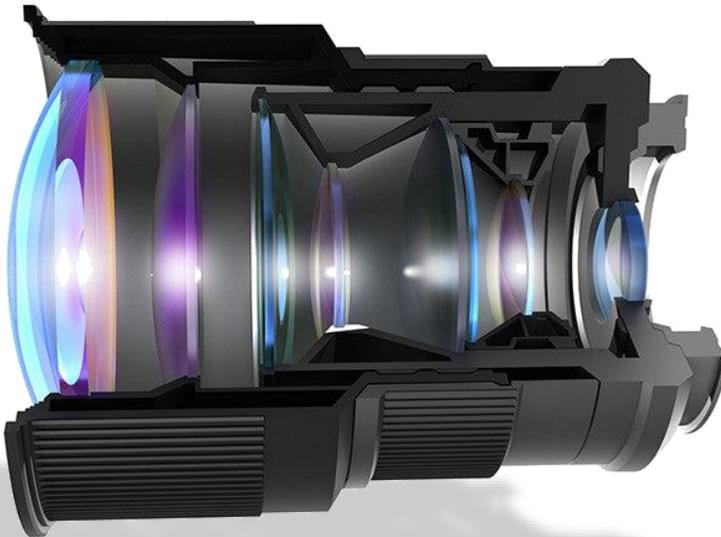
Rifacendo la trasformata applicando questo «filtro» ottengo la curva nera

# Diffrazione e Risoluzione Ottica

→Questo significa che se voglio fare imaging della sorgente locale confinata dovrò scontrarmi con questo «filtro» in frequenze spaziali che determina una resa diversa



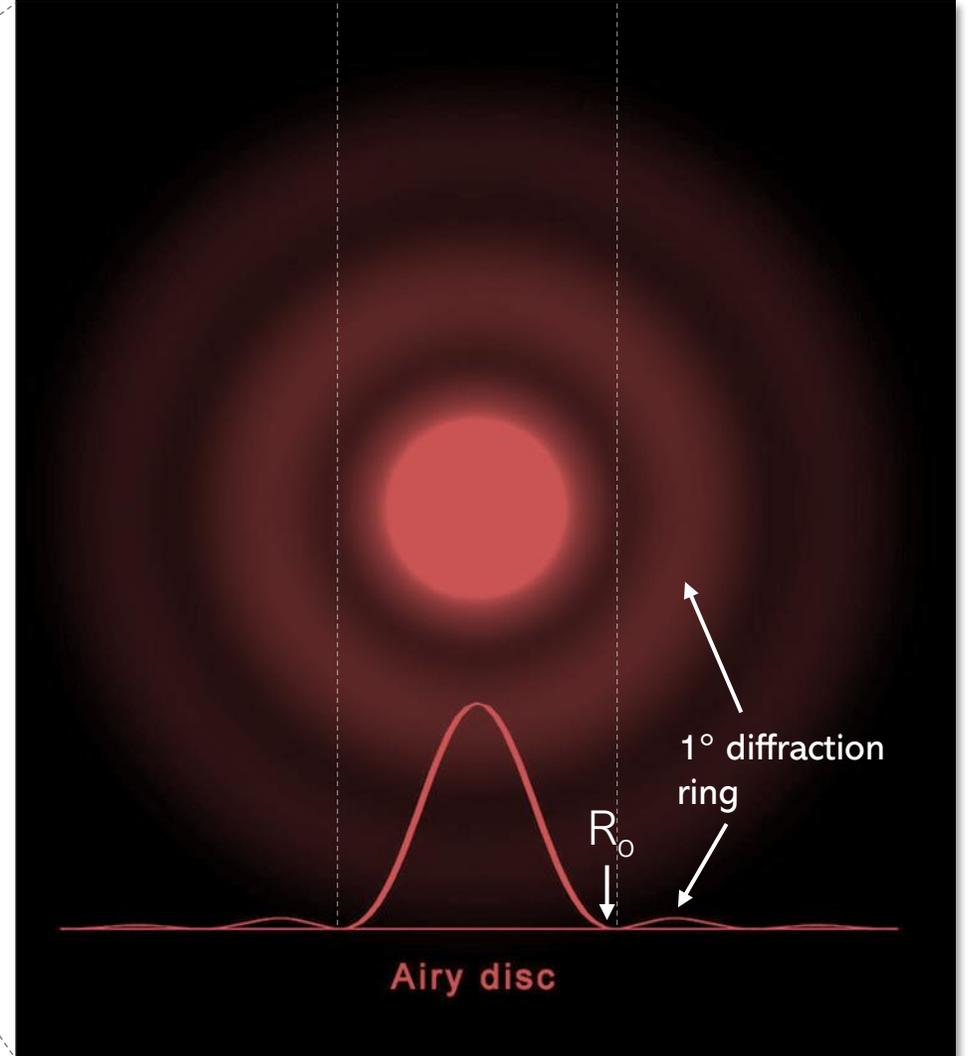
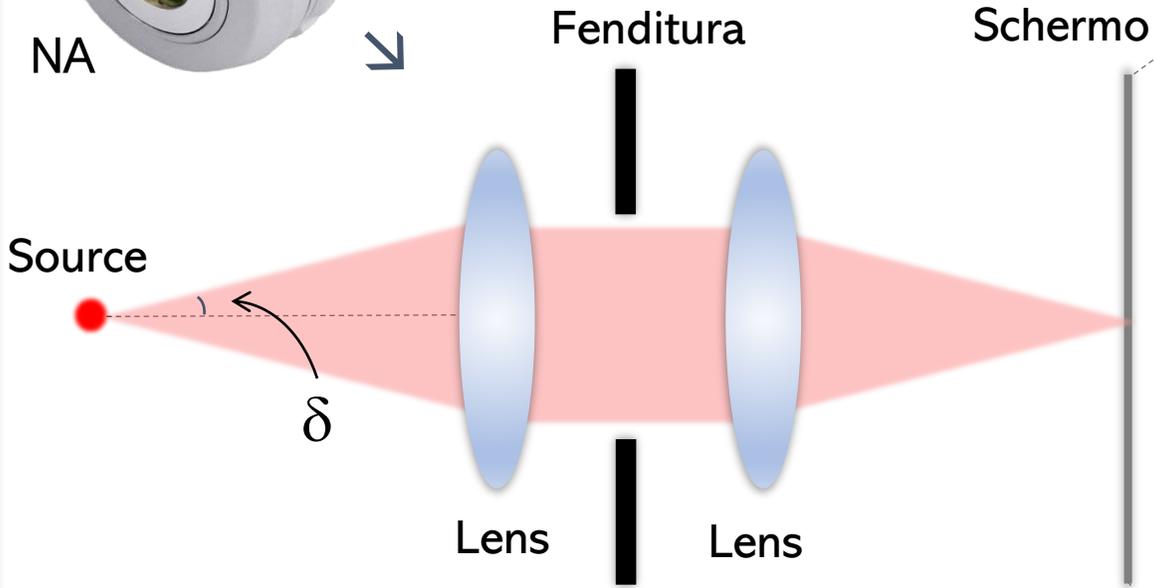
LIMITE DIFFRATTIVO DELLE LENTI



# Diffrazione e Risoluzione Ottica



NA

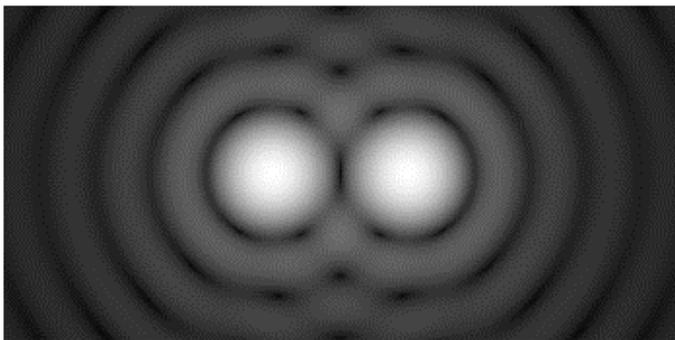


$$R_0 = \frac{0,61 \lambda}{n \sin(\delta)} = \frac{0,61 \lambda}{NA}$$

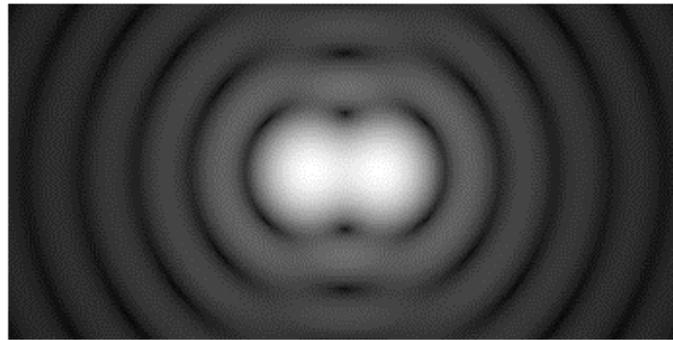
NA: Apertura numerica dell'obiettivo

# Diffrazione e Risoluzione Ottica

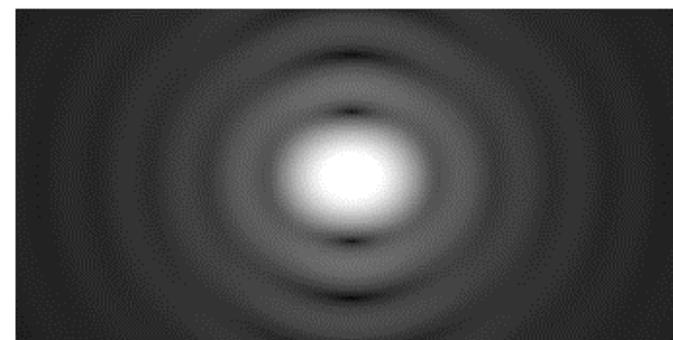
Criterio di Rayleigh



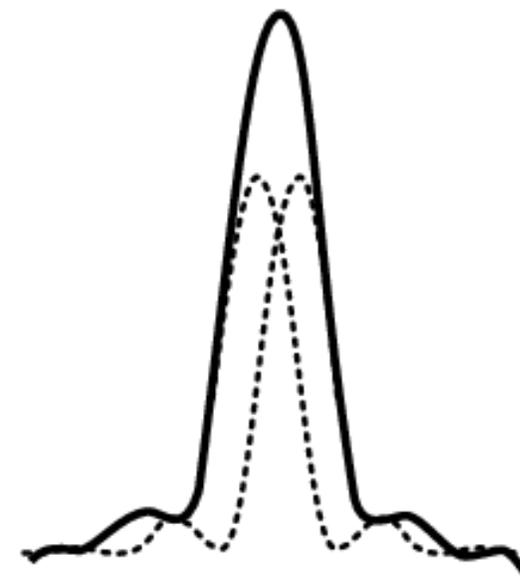
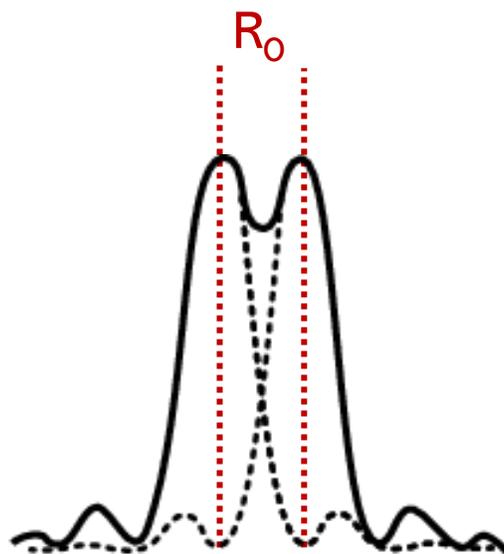
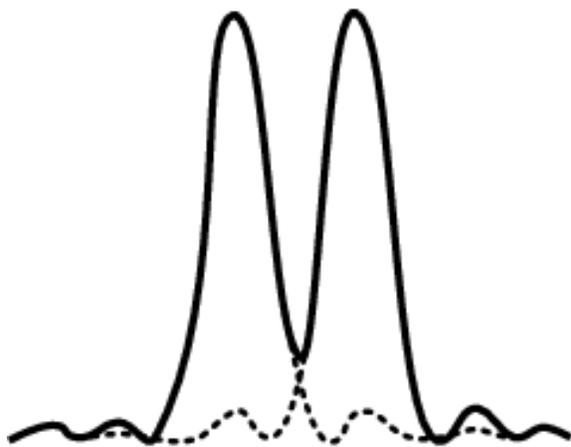
Resolved



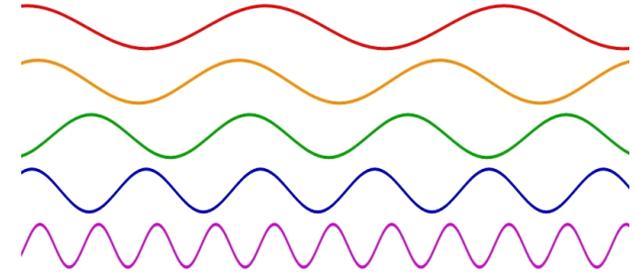
$R_0$



Unresolved



# Risoluzione Ottica obiettivi



NA: 0.3 – 0.9



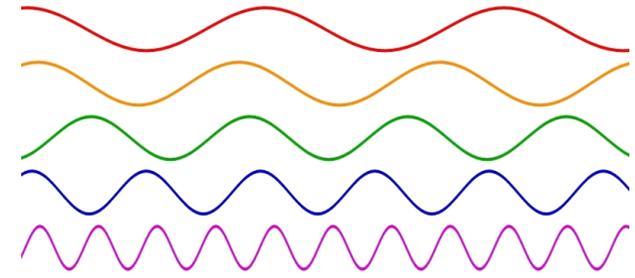
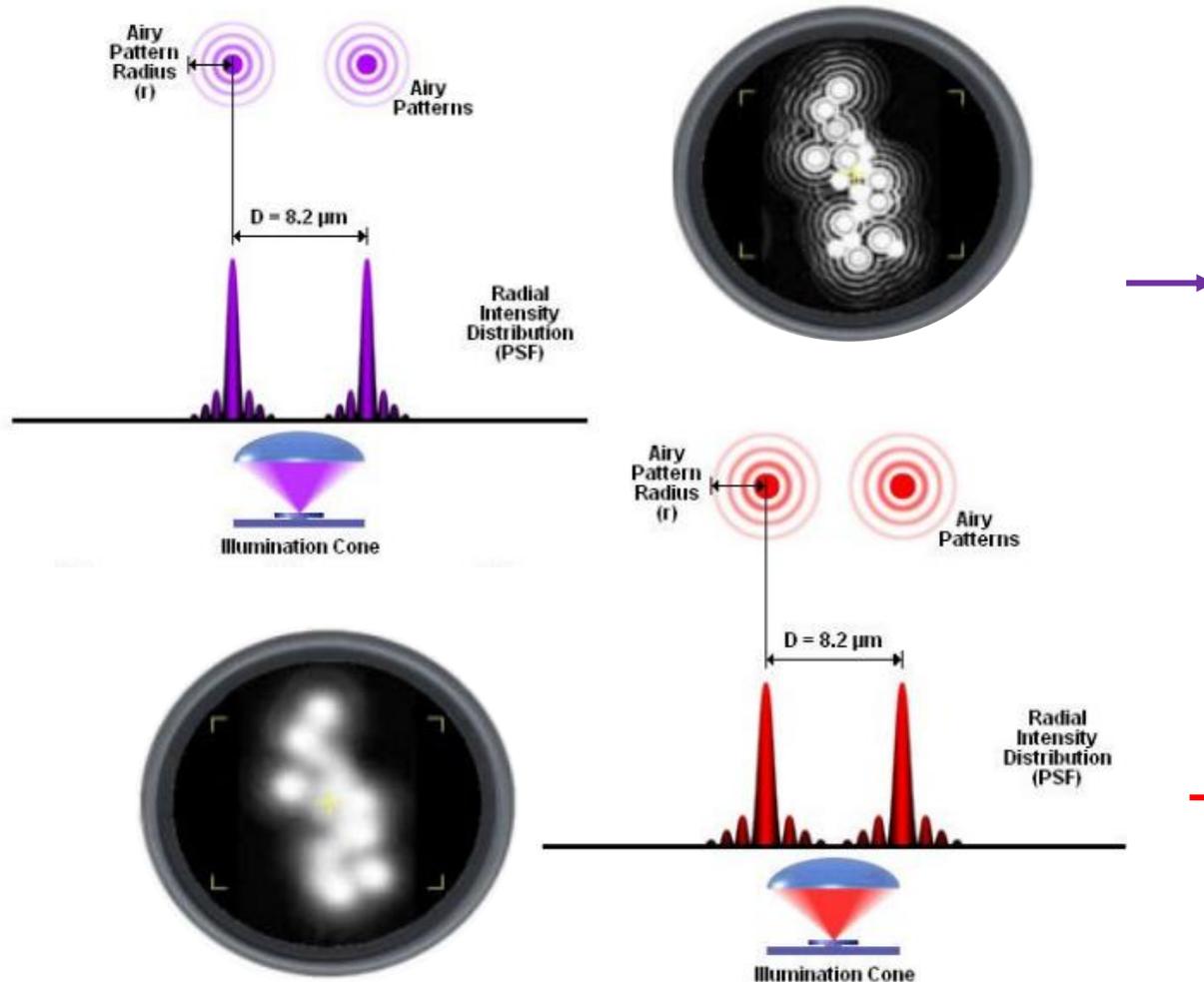
$$R_0 = \frac{0,61 \lambda}{NA}$$

Lunghezza d'onda (nm)	Risoluzione ottica (nm)
350	153
400	174
450	196
500	218
550	240
600	261
650	283
700	305

Rule of thumb  $\rightarrow R_0 \approx \lambda/2$

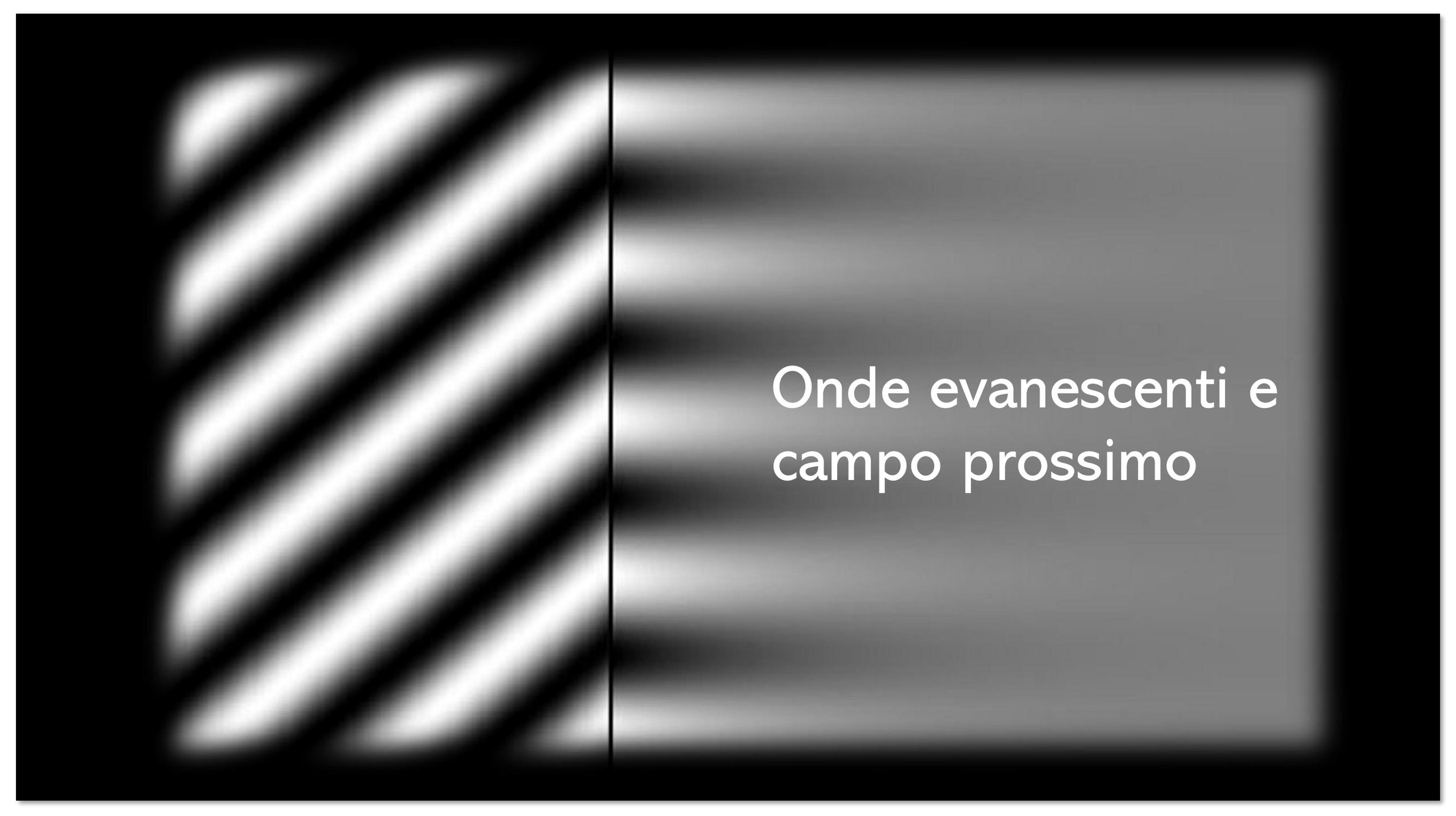
# Risoluzione Ottica obiettivi

A parità di NA, a  $\lambda$  minori  $R_0$  è minore e quindi si possono distinguere dettagli spaziali più piccoli



Lunghezza d'onda (nm)	Risoluzione ottica (nm)
350	153
400	174
450	196
500	218
550	240
600	261
650	283
700	305

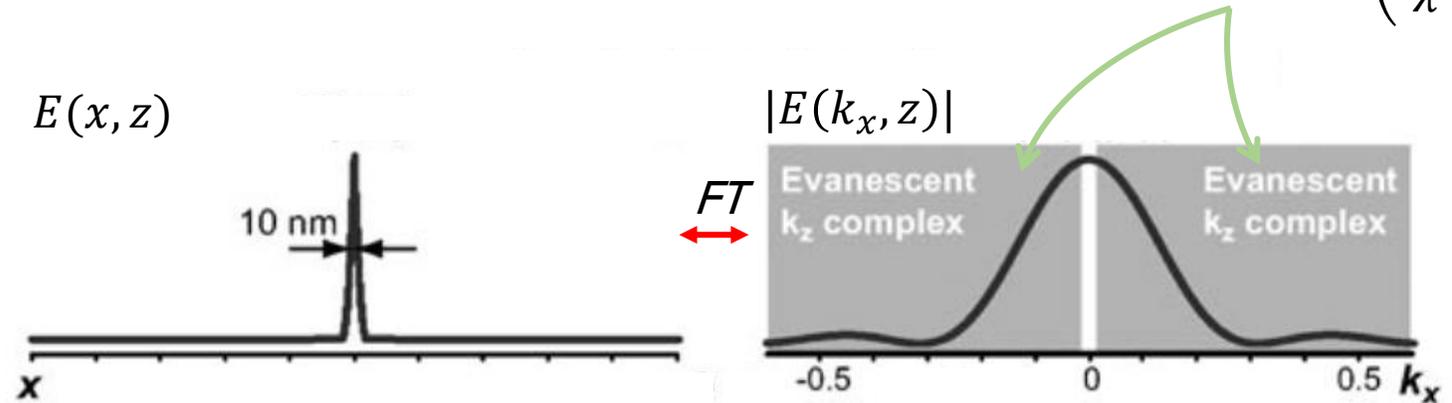
Rule of thumb  $\rightarrow R_0 \approx \lambda/2$



Onde evanescenti e  
campo prossimo

# Onde Evanescenti

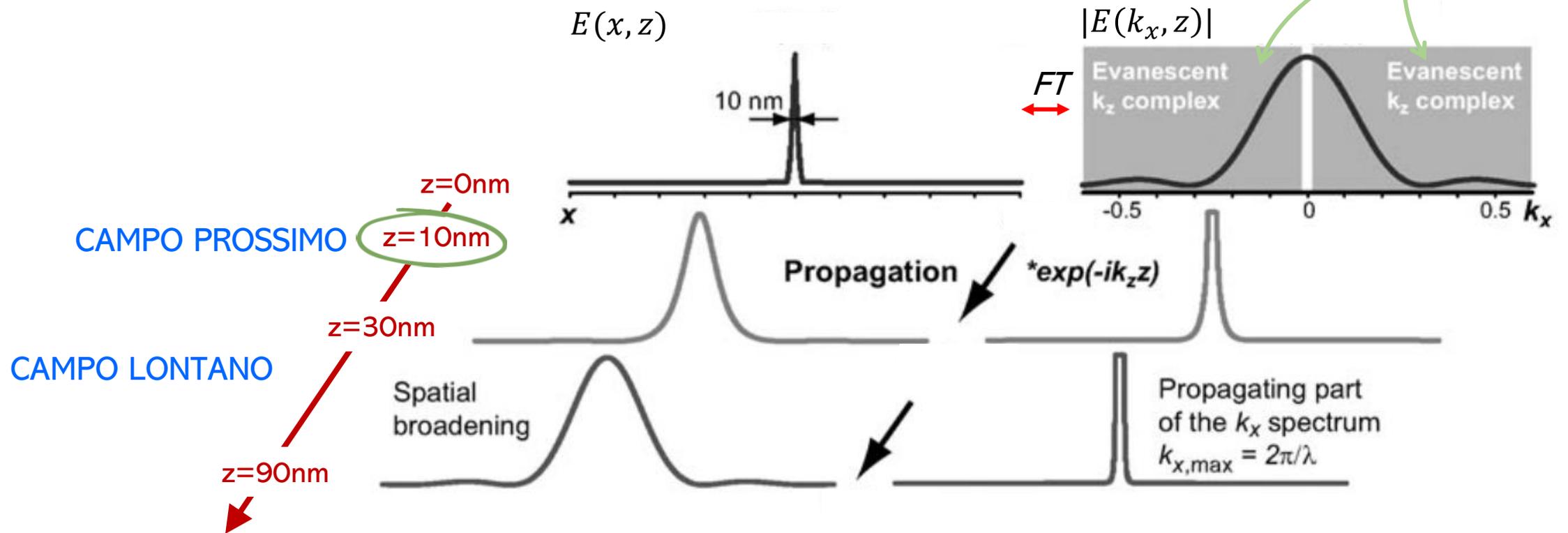
Esistono delle soluzioni delle Equazioni di Maxwell per le quali il filtro non c'è:  $|k_x|^2 \geq \left(\frac{2\pi}{\lambda}\right)^2$



Grazie al particolare comportamento delle componenti  $k_z$ , queste soluzioni, dette onde evanescenti, decadono esponenzialmente su distanze  $\approx \Delta x/2$

# Onde Evanescenti

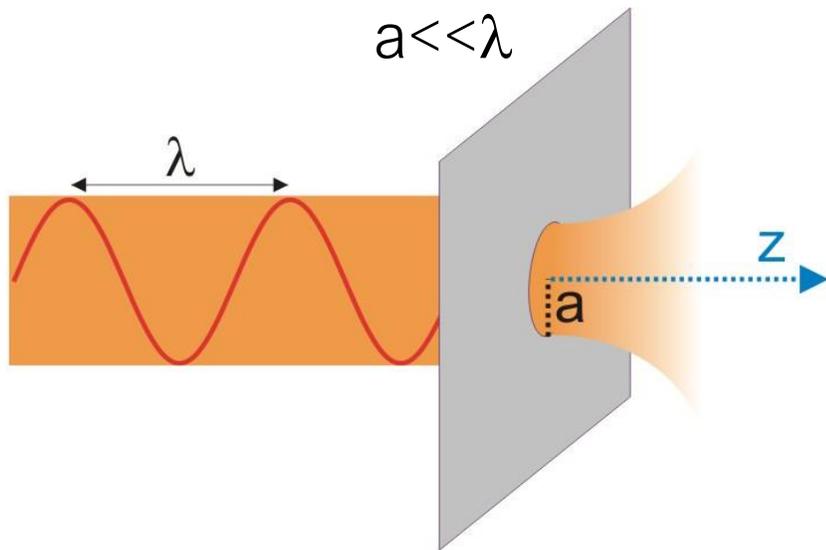
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Grazie al particolare comportamento delle componenti  $k_z$ , queste soluzioni, dette onde evanescenti, decadono esponenzialmente su distanze  $\approx \Delta x/2$

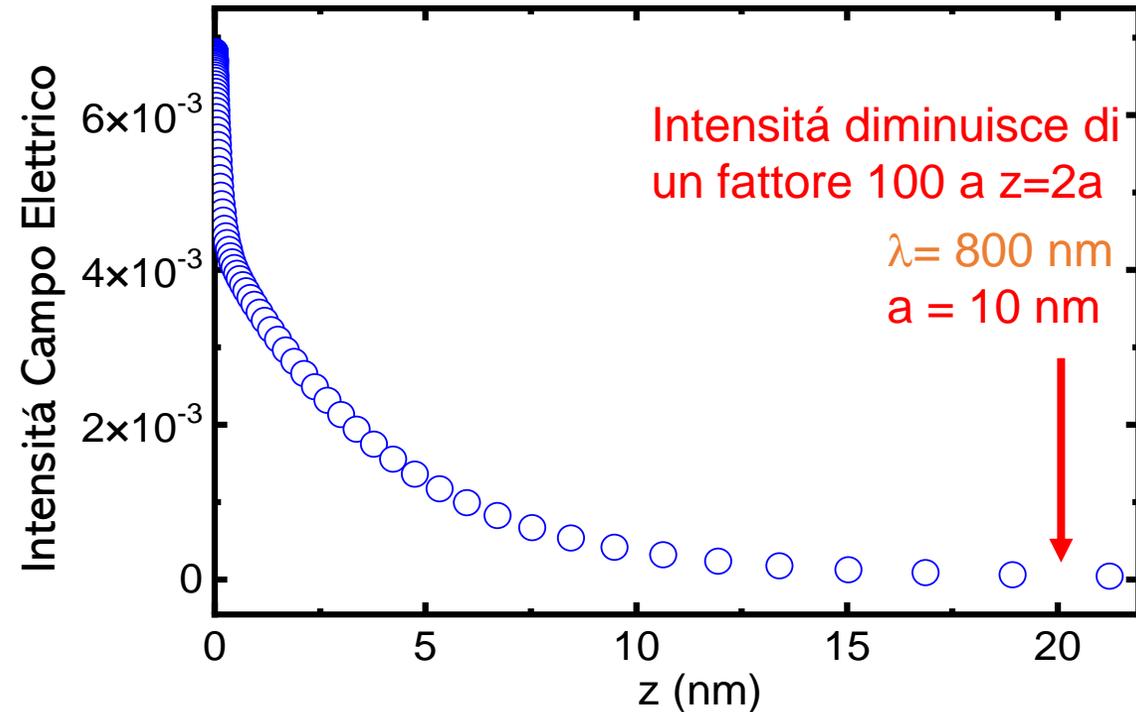
# Onde Evanescenti

L'ottica ondulatoria classica, ha un limite fondamentale perché non considera il ruolo delle onde evanescenti nei fenomeni di diffrazione. Infatti, le soluzioni classiche dei problemi di diffrazione sono corrette nella zona radiativa (lontana dalla sorgente), ma non sono in grado di riprodurre ciò che succede nelle vicinanze della sorgente.

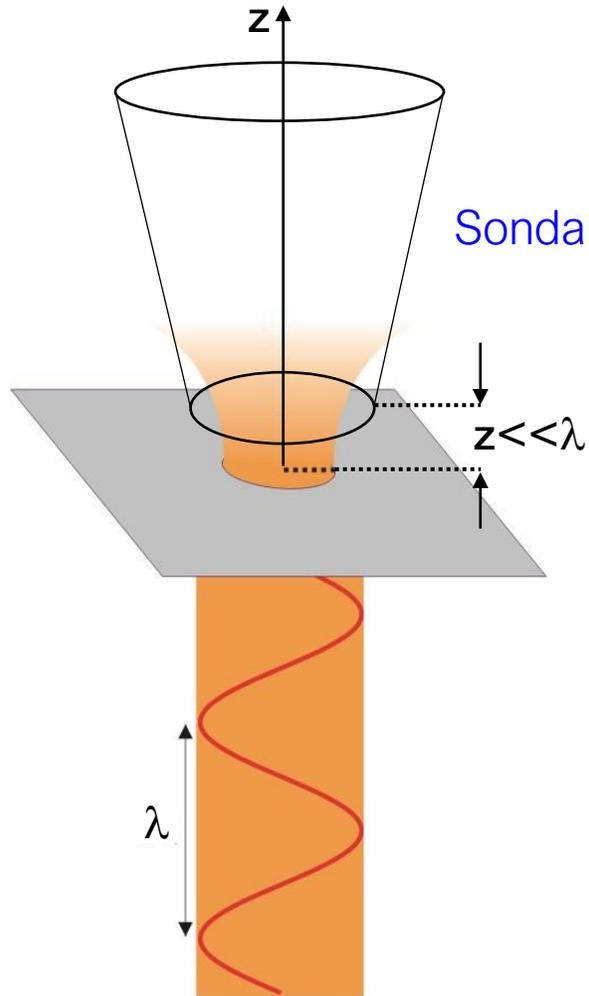


Il campo evanescente rimane confinato entro una distanza circa pari al raggio della fenditura.

Campo elettrico lungo  $z$

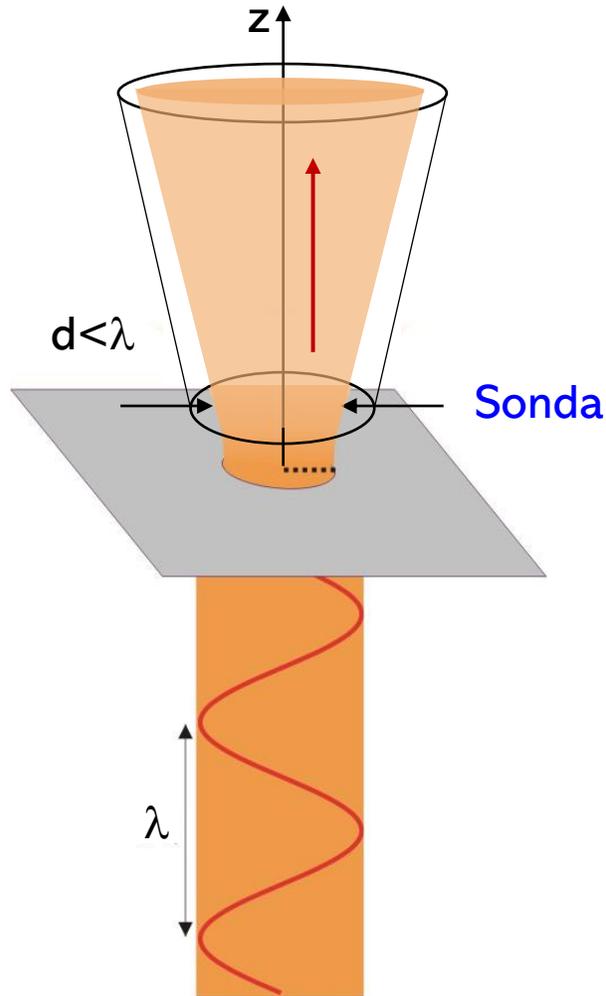


# Microscopia in Campo Prossimo



L'idea centrale della microscopia in campo vicino è quella di migliorare la risoluzione spaziale attingendo alle componenti evanescenti, e quindi utilizzando una sonda nel campo prossimo.

# Microscopia in Campo Prossimo



Campo lontano: risoluzione  $\rightarrow$  limite diffrattivo

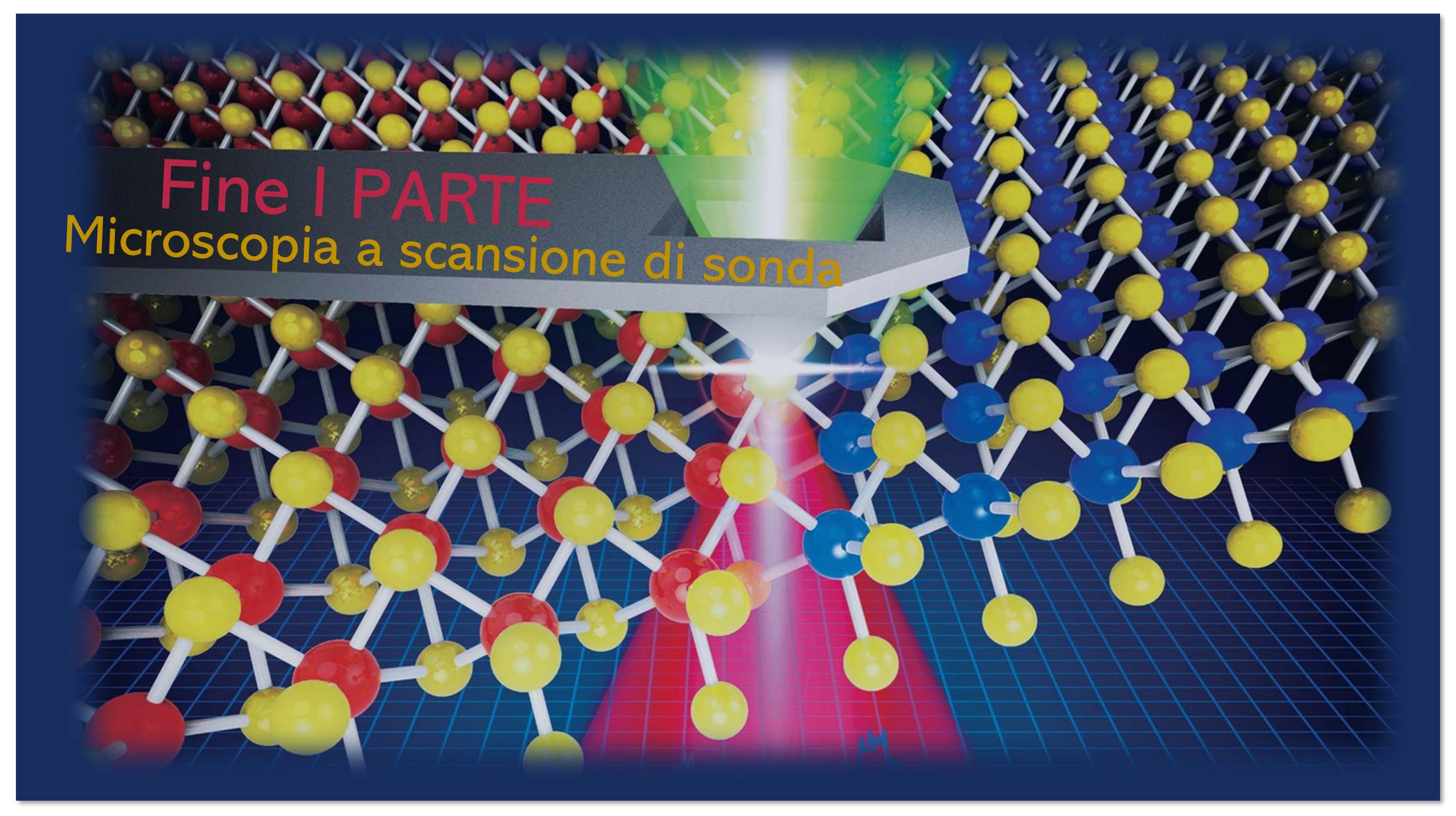
$$R_0 \approx \lambda/2$$

*Un modo per superare il limite di diffrazione*

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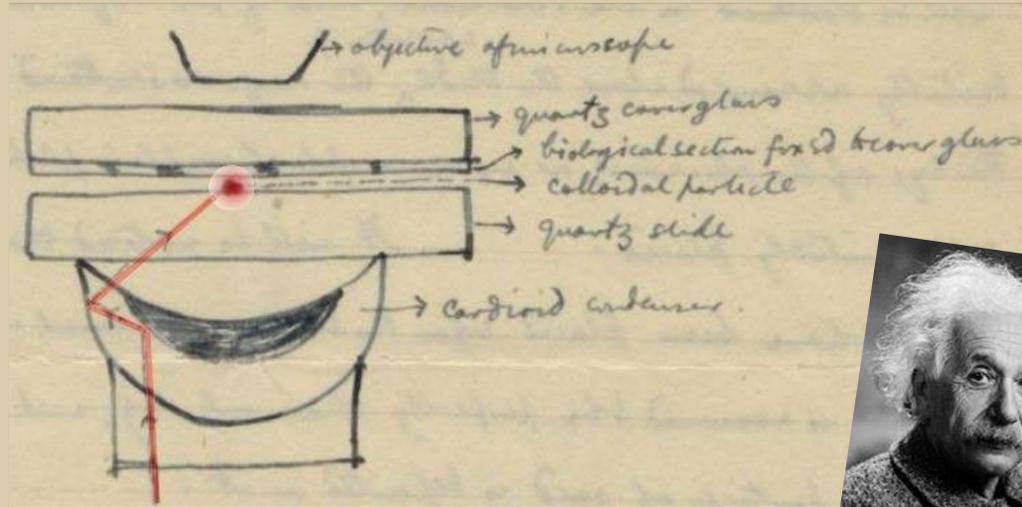
*Campo prossimo: risoluzione  $\rightarrow$  dimensione della sonda*



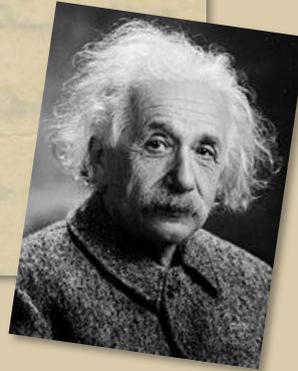
**Fine I PARTE**  
Microscopia a scansione di sonda

# 1928, Edward Synge

Storicamente il primo ad avere avuto l'idea di sfruttare il campo prossimo per superare il limite della diffrazione



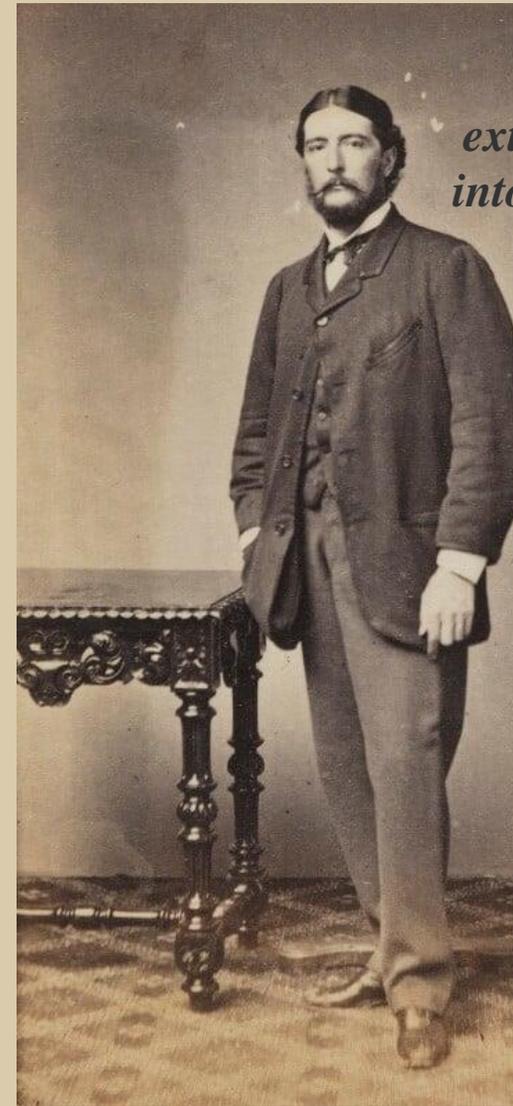
Lettera ad Albert Einstein, 1928



«Aperture»

«Scanning»

«Ultra-microscopy»



*“A suggested method for extending microscopic resolution into the ultra-microscopic region”*

356 Mr. E. A. Synge on a Method  
line  $2^2p_0-1(S_0)$ , that increase with the first power of the exciting light.  
We saw at the beginning that Wood had found that the application of a magnet to the arc increased 2537 four times, 3654 eight times, and 3650 sixteen times. It is now possible to explain those changes. The four-fold increase of 2537 shows that the reversal of that line in the arc has been reduced by the magnet so as to increase four times the intensity of the core of the line. Only the core of the line is absorbed by the vapour at room temperature. This increase of 2537 brings four times as many atoms to the level  $2^2p_1$ . The line 3654 appears when no foreign gases are present, mainly as a result of the absorption of 3125. The fact that 3654 increases not only four times but eight shows that the intensity in the core of 3125 has been increased two times by the magnet. The same is true for 3650. The reversal of 3125 and 3650 in the arc without the magnetic field seems, then, to be about half as strong as the reversal of the resonance line 2537. This result is quite plausible.

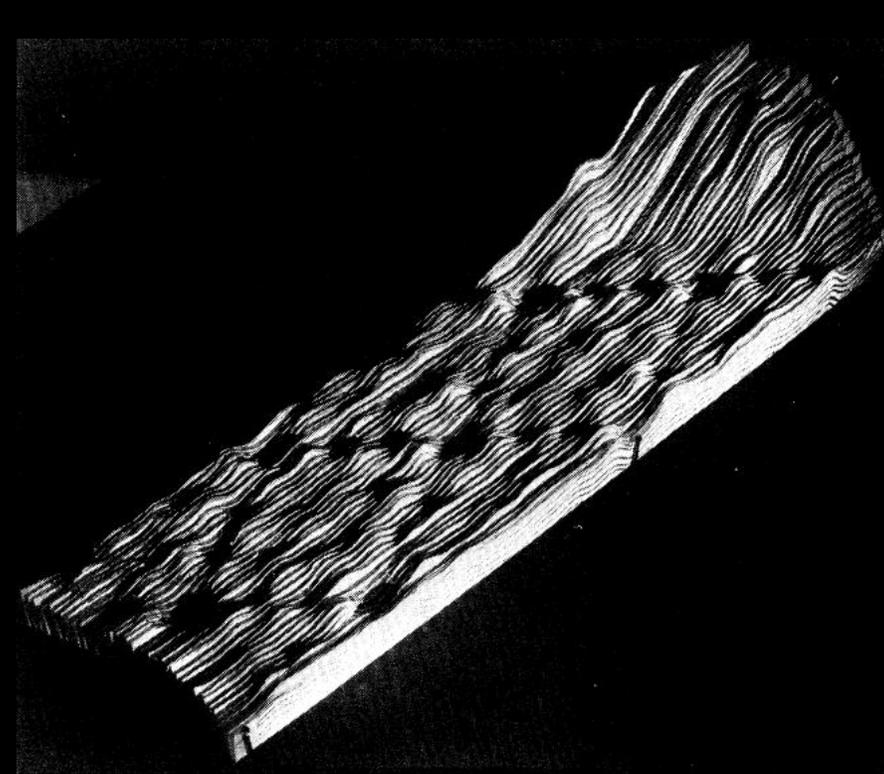
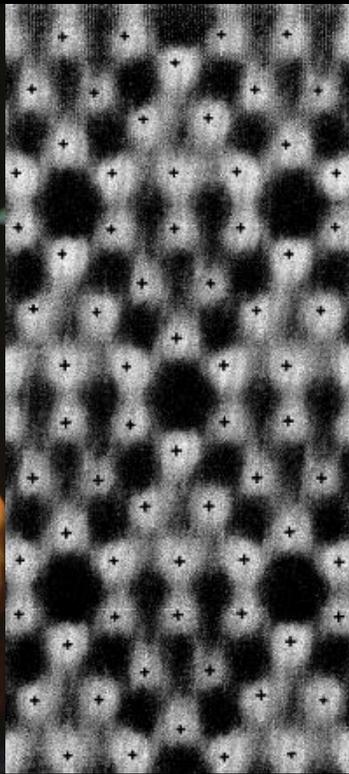
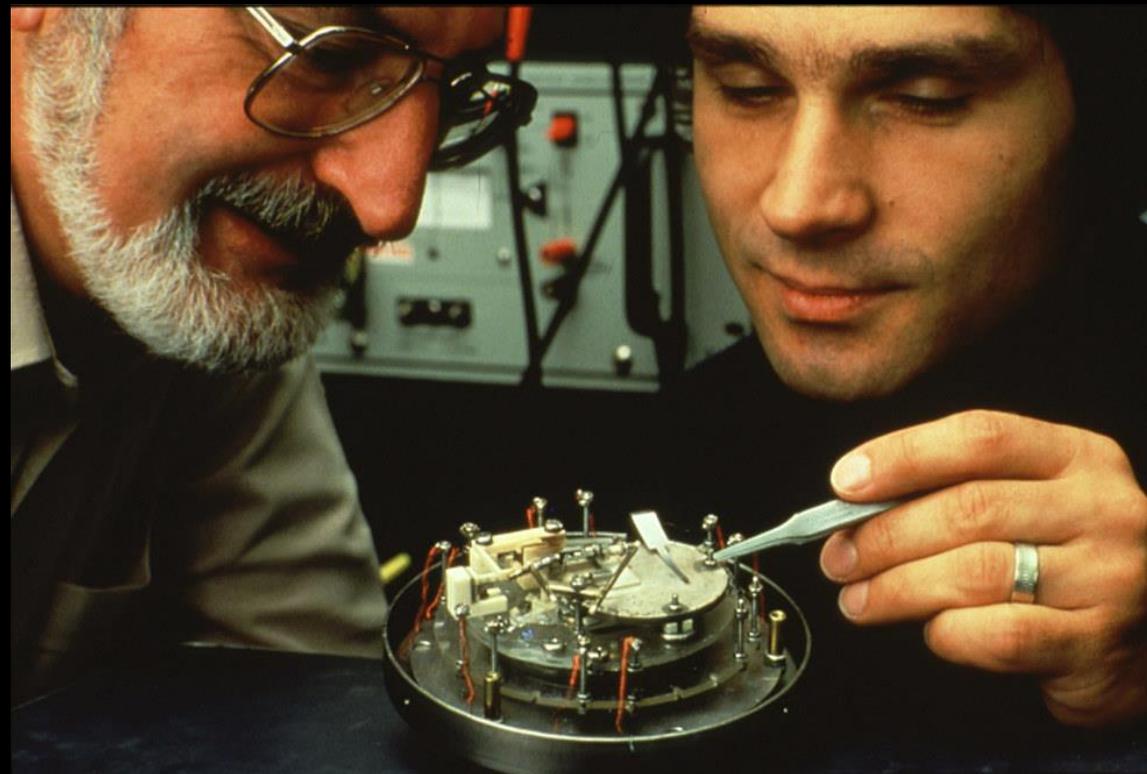
XXXVIII. *A Suggested Method for extending Microscopic Resolution into the Ultra-Microscopic Region.* By E. H. SYNGE\*.

IT is generally accepted as an axiom of microscopy that the only way to extend resolving-power lies in the employment of light of smaller wave-lengths. Practical difficulties, however, rapidly accumulate as light of increasingly small wave-length is brought into service, and probably little hope is entertained of arriving at a resolution much beyond  $\cdot 1 \mu$ , with, perhaps,  $\cdot 05 \mu$  as an extreme limit.  
Yet a method offers itself which lies a little outside the beaten track of microscopic work and raises various technical problems of a new kind, but which makes the attainment of a resolution of  $\cdot 01 \mu$ , and even beyond, dependent upon a technical accomplishment which does not seem impracticable at present. The idea of the method is exceedingly simple, and it has been suggested to me by a distinguished physicist that it would be of advantage to give it publicity, even though I was unable to develop it in more than an abstract way.

\* Communicated by the Author.



Nobel Prize in Physics 1986

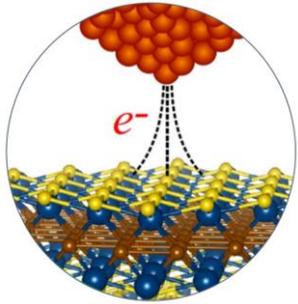


G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, Phys. Rev. Lett. **49**, 57, (1982)

Microscopia a Effetto Tunnel

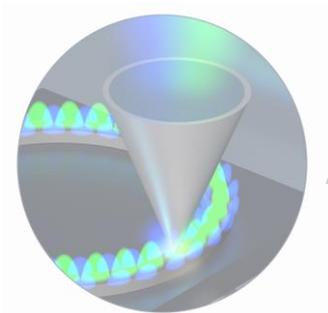
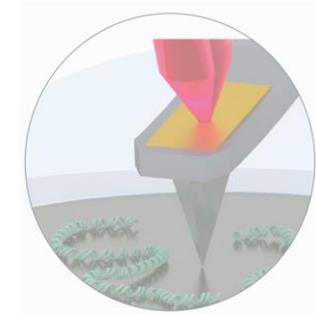
# Microscopie in campo prossimo

Tutte le tipologie di microscopie a scansione di sonda in campo vicino sono basate sullo scanning della sonda sulla superficie del campione monitorando l'interazione (qualunque essa sia) tra sonda e superficie stessa.



Scanning Tunneling Microscopy (STM)

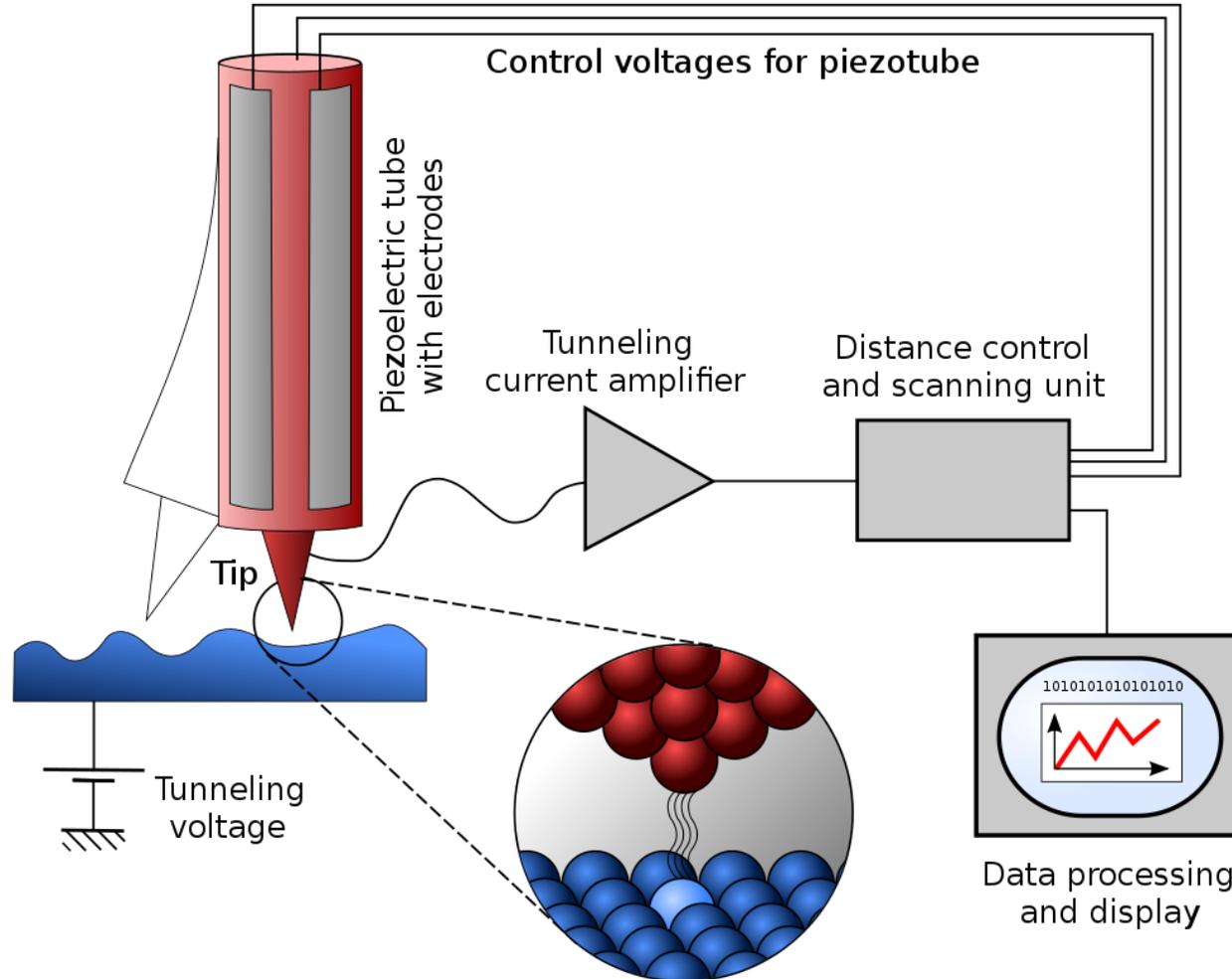
Atomic Force Microscopy (AFM)



Aperture Scanning Near-field Optical Microscopy (a-SNOM)

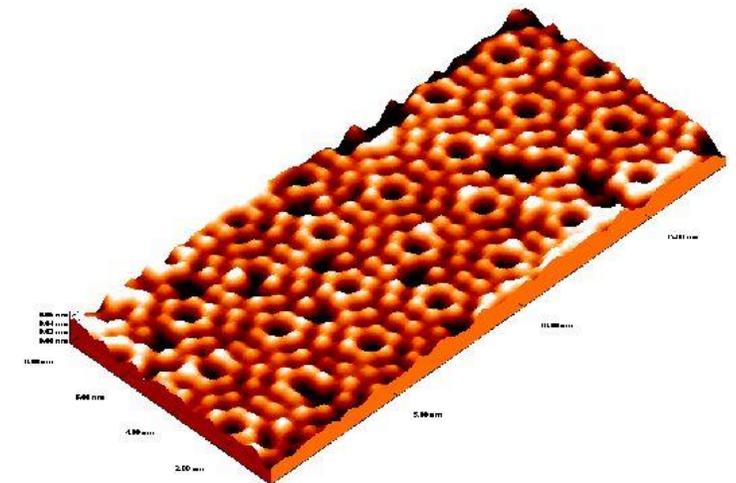
# Microscopio a scansione a effetto tunnel

Il microscopio a effetto tunnel è un potente strumento per lo studio delle superfici a livello atomico, basato sull'effetto quantistico di tunneling tra gli elettroni del campione alla sonda quando viene applicato un Voltaggio.



1981 invenzione della  
**Scanning Tunneling Microscopy (STM)**

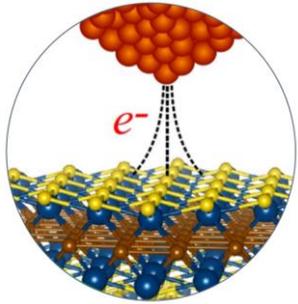
0.1 nm risoluzione laterale e 0.01 nm  
risoluzione in profondità



Si(111)7×7 ricostruzione, 18×8 nm<sup>2</sup>

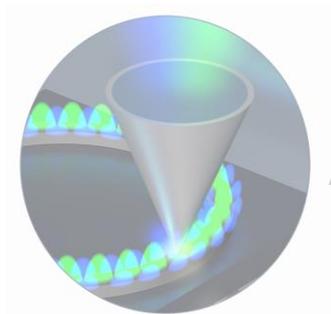
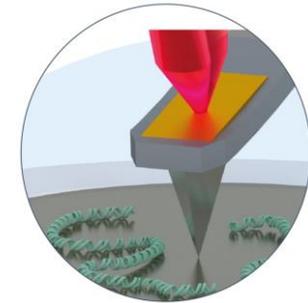
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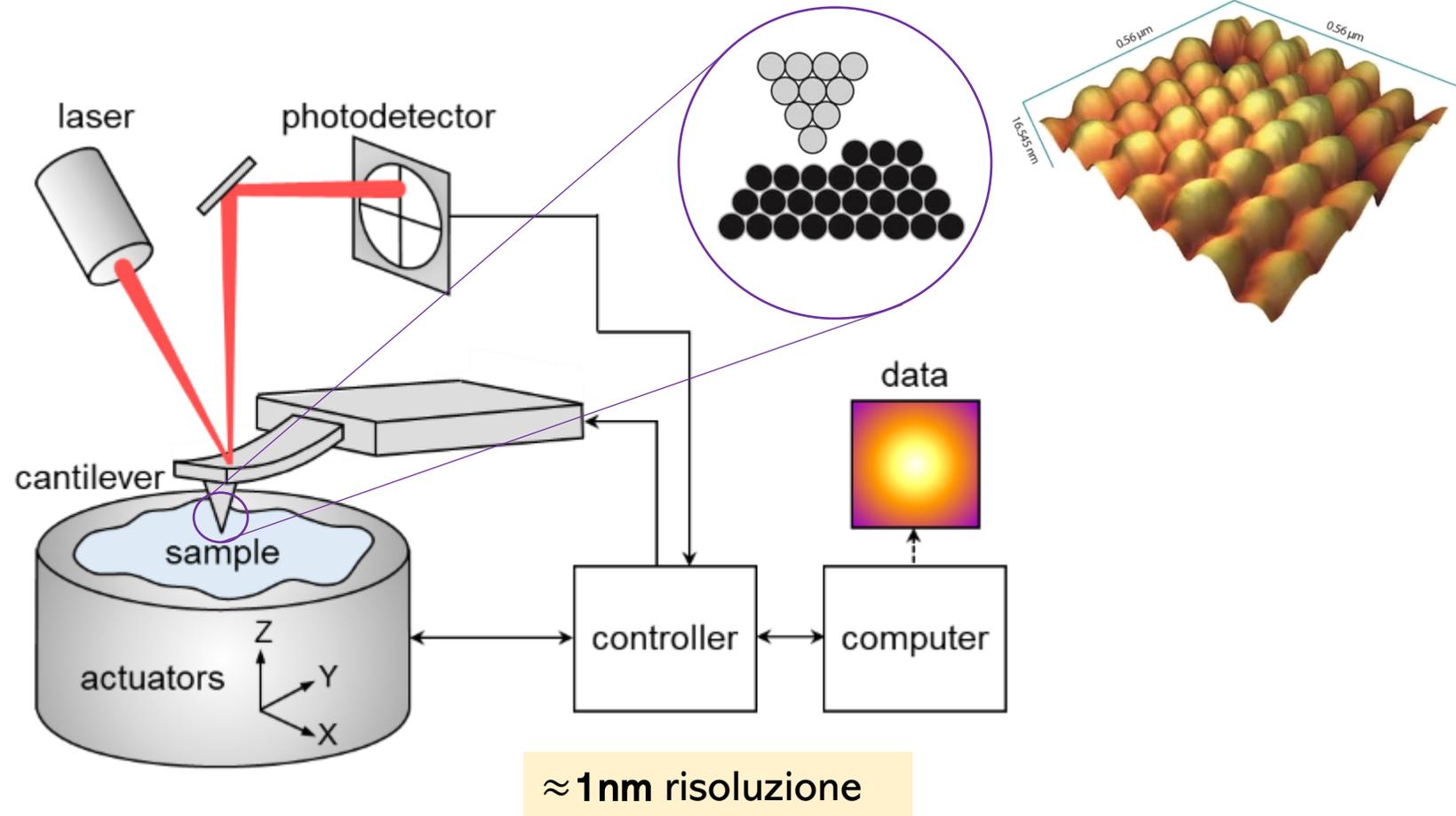
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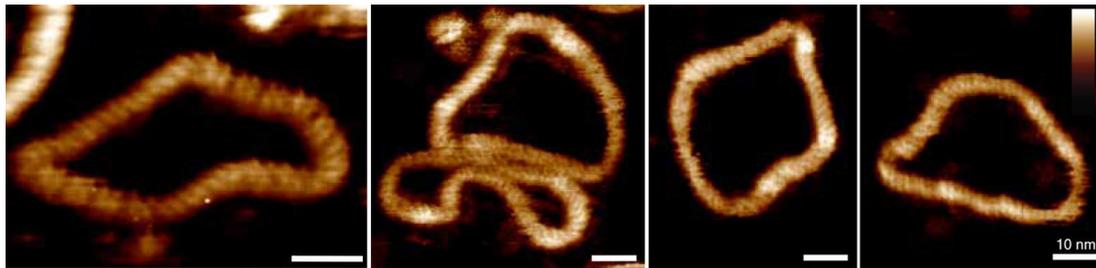
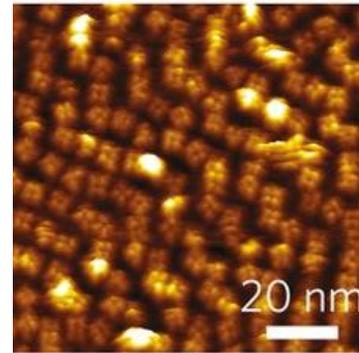
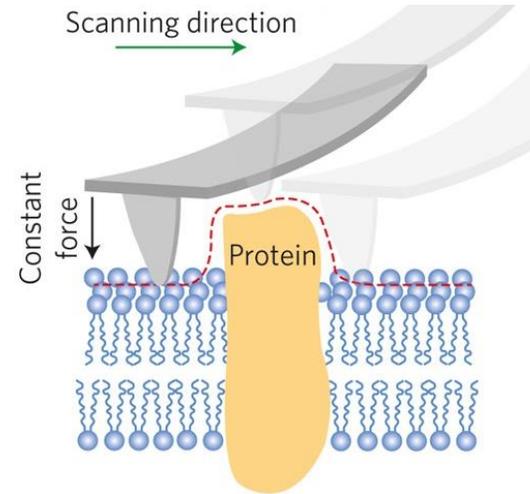
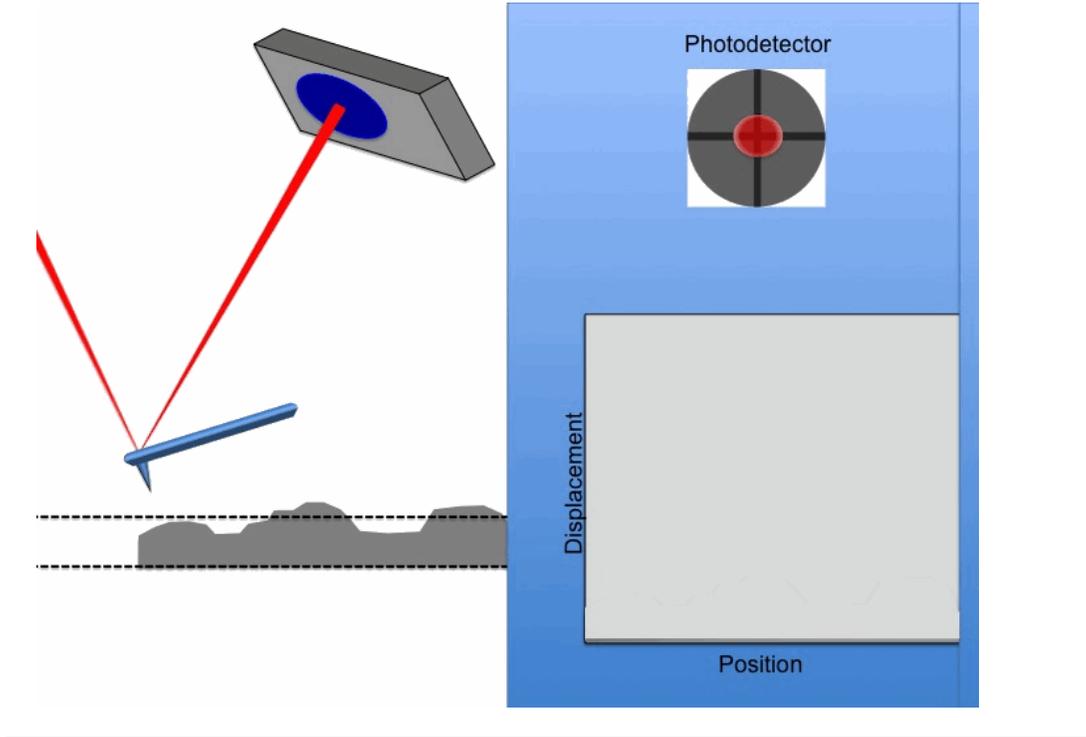
Aperture Scanning Near-field Optical Microscopy (a-SNOM)

# Microscopio a forza atomica (AFM)

Il microscopio a forza atomica é lo sviluppo dell'STM, e sfrutta le forze di *Van der Waals* tra sonda e campione.



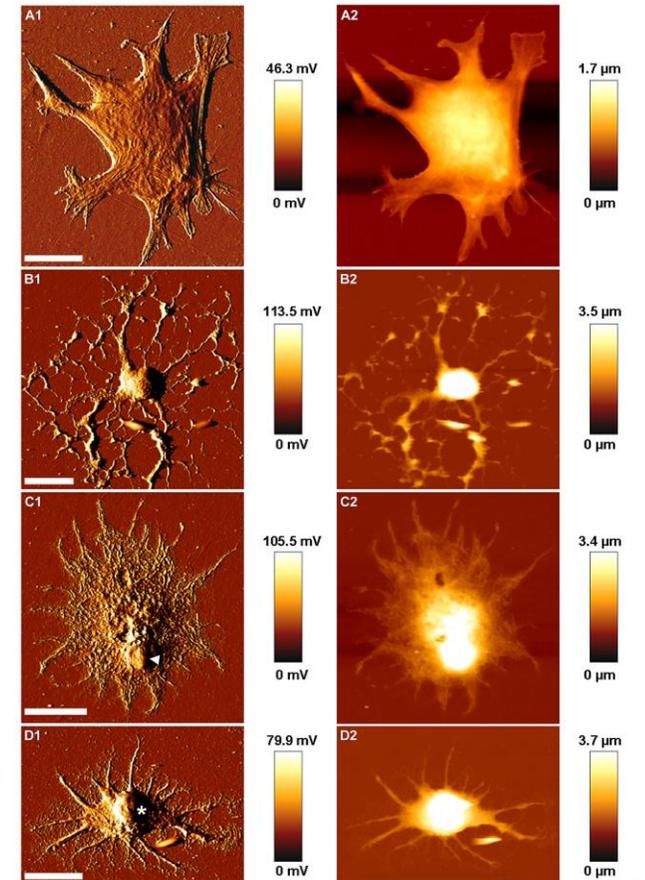
# Microscopio a forza atomica (AFM)



DNA circles - A. L. B. Pyne et al. , Nat. Comm., 12, 1053 (2021)

Maggiore uso ne viene fatto per campioni di tipo biologico

Neurons and Glial cells  
O. Kaiser et al., PLoS ONE 8(12): e80490 (2013)

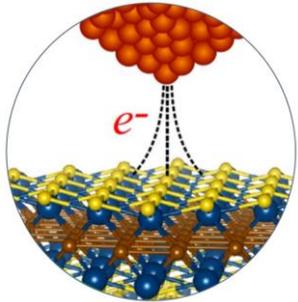


Cyclic nucleotide-regulated potassium channels (MlotiK1) reconstituted into lipid membranes

Y. Dufrene et al. Nat. Nanotech. 12, 295–307 (2017)

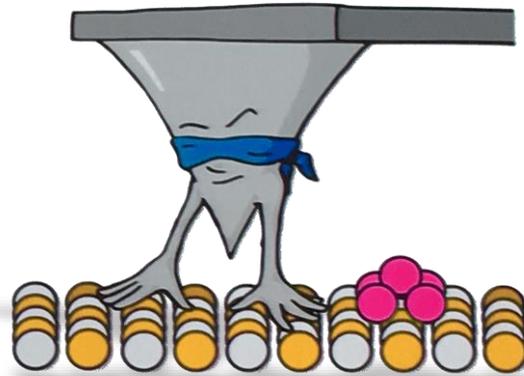
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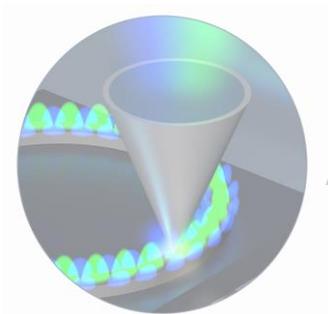
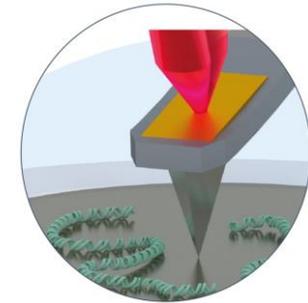
Scanning Tunneling Microscopy (STM)

Nessun segnale ottico!



©Markus B. Raschke

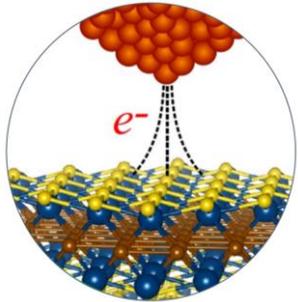
Atomic Force Microscopy (AFM)



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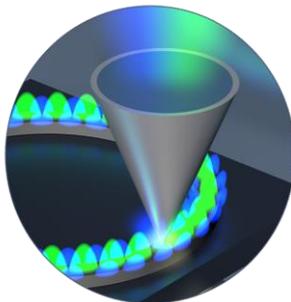
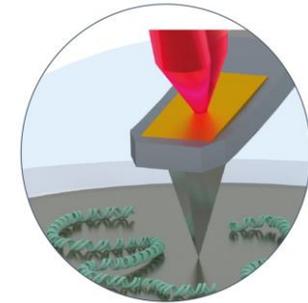
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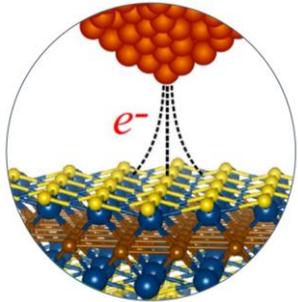
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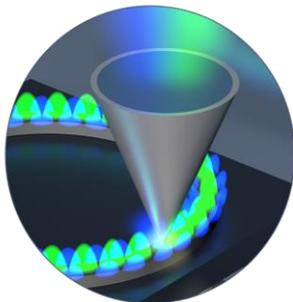
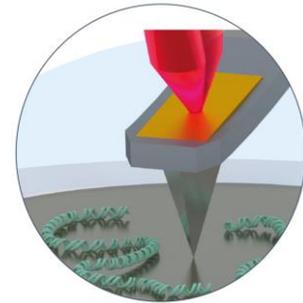
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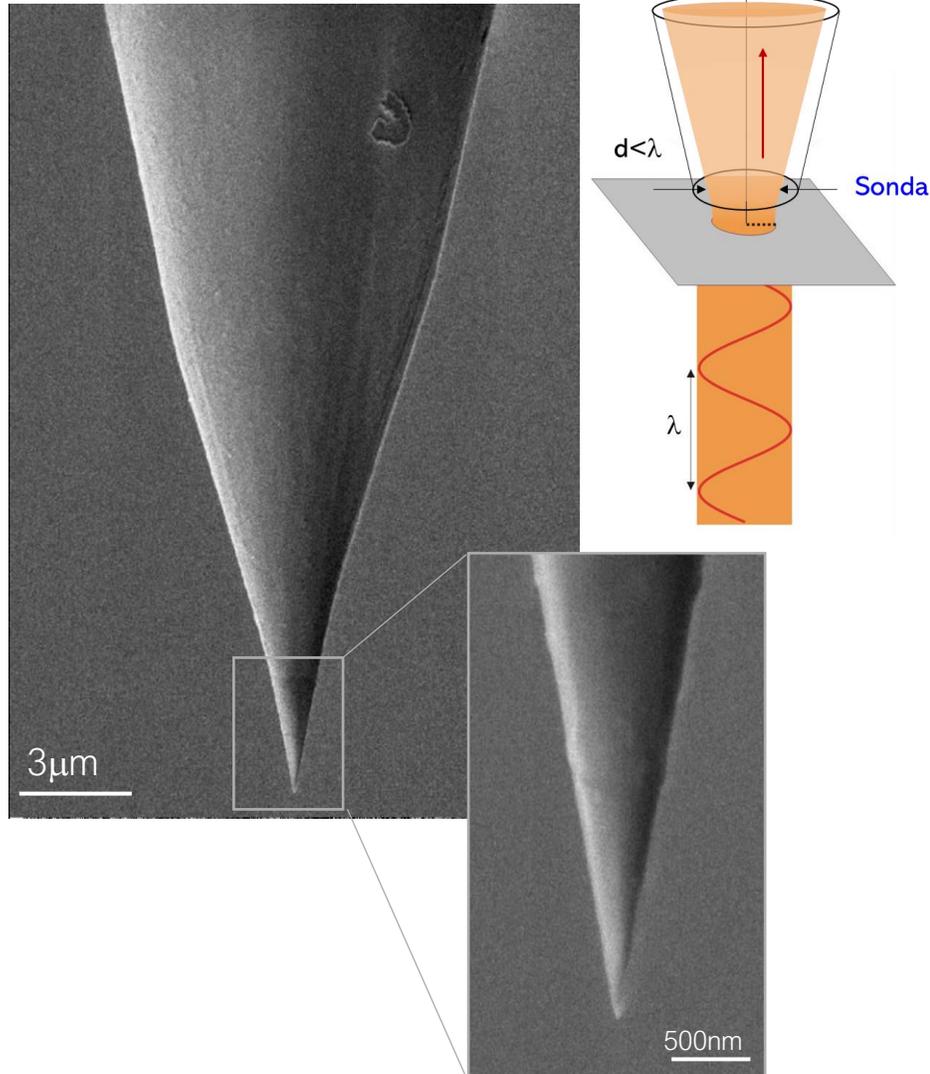
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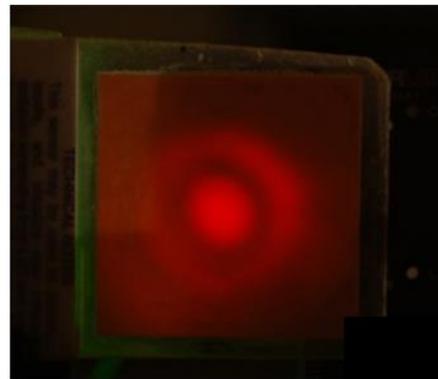
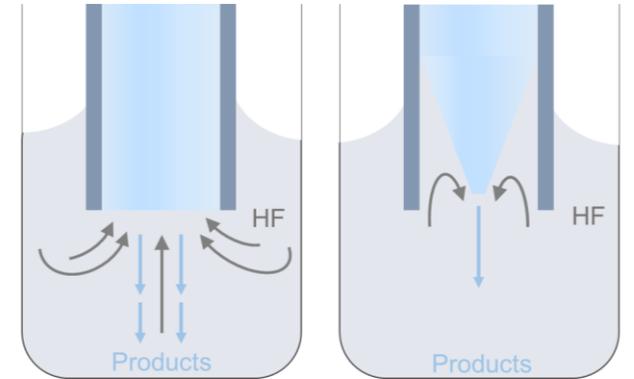
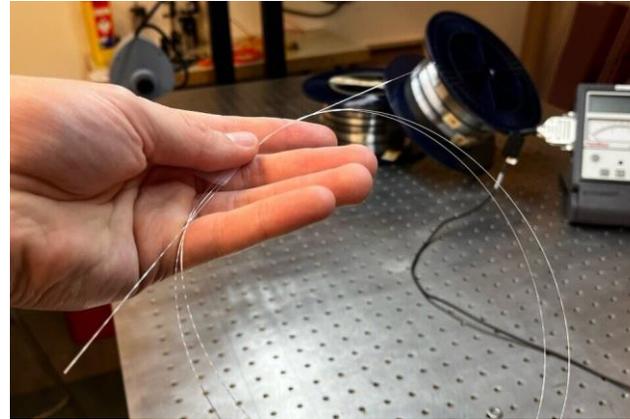
Aperture Scanning Near-field Optical Microscopy (a-SNOM)

# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)

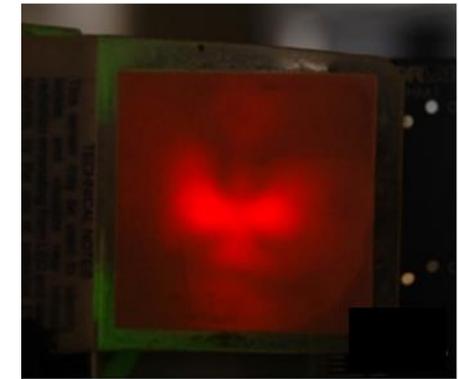
Punta dielettrica da fibra ottica



Metodo di etching chimico

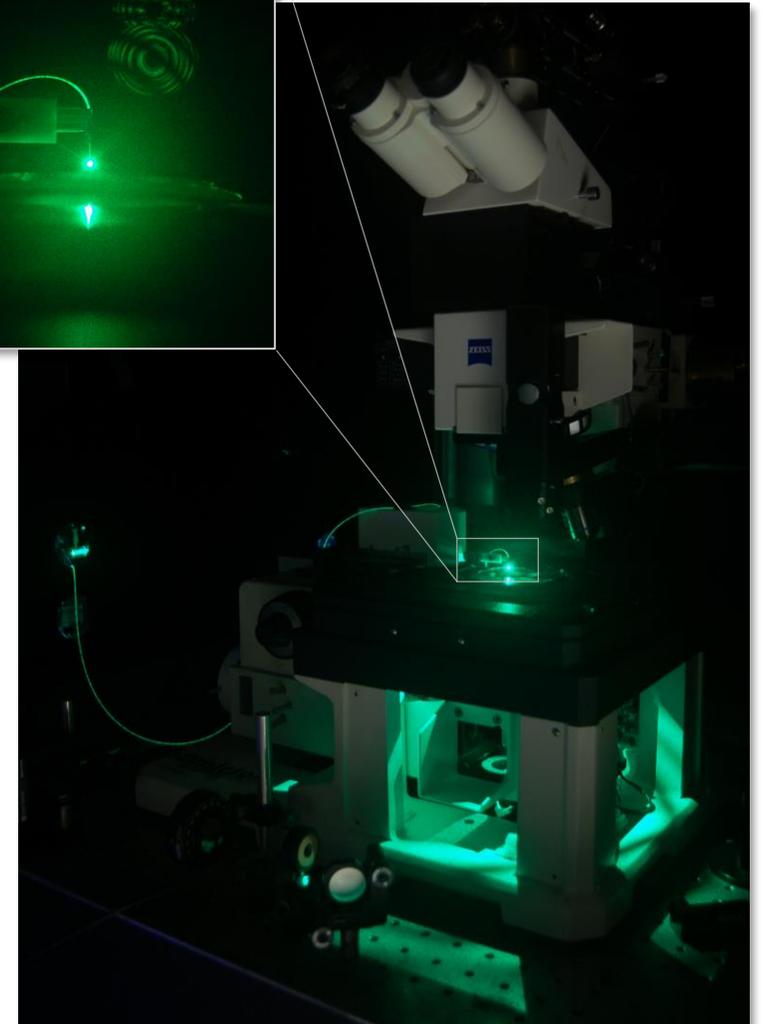
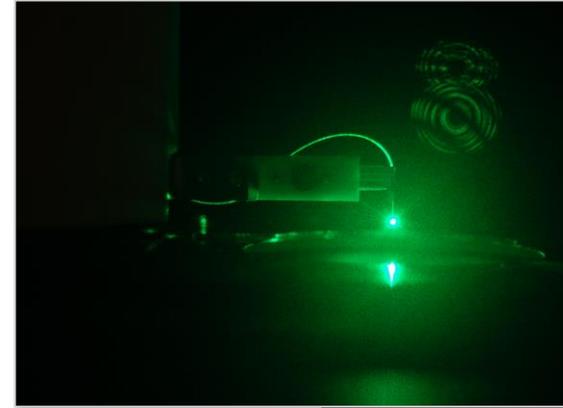
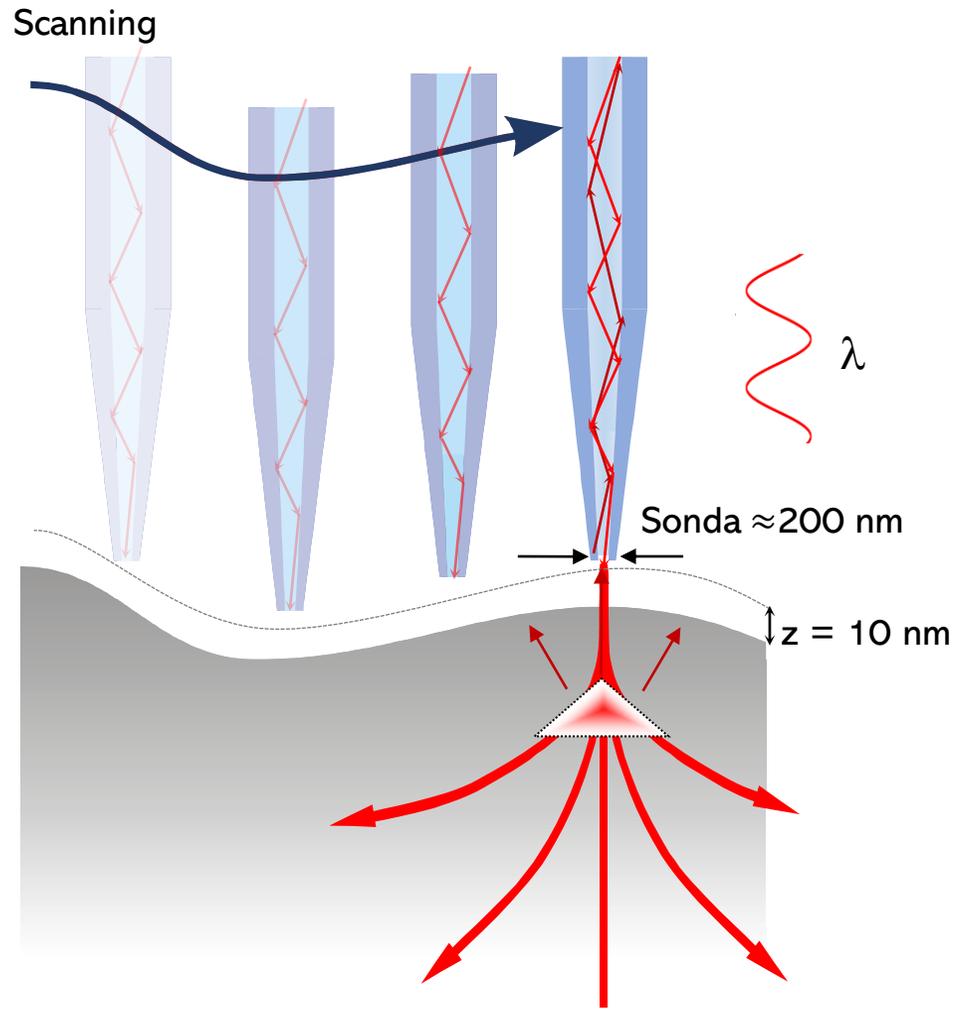


*Trasmissione da fibra funzionante*



*Etching non buono*

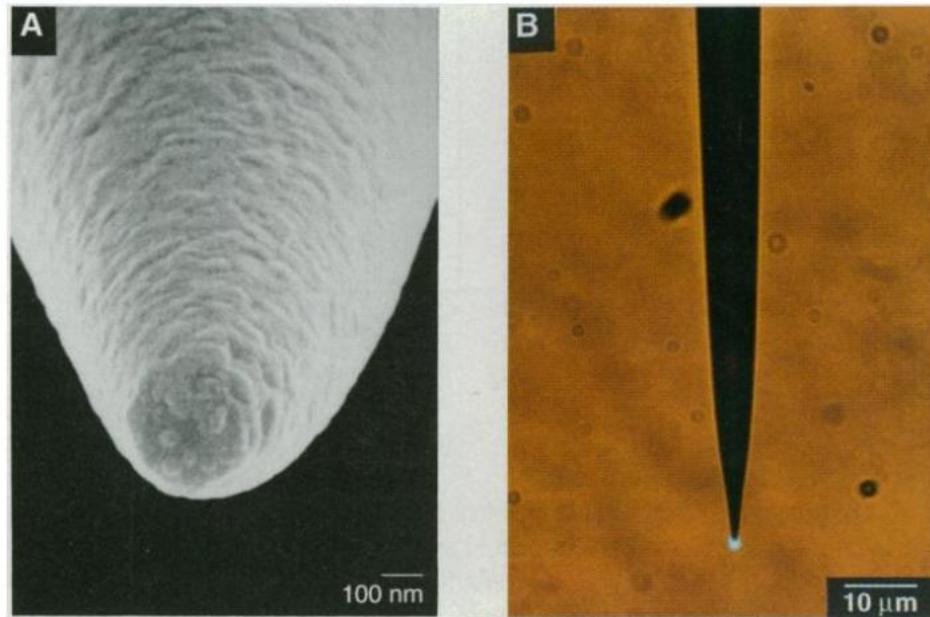
# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)



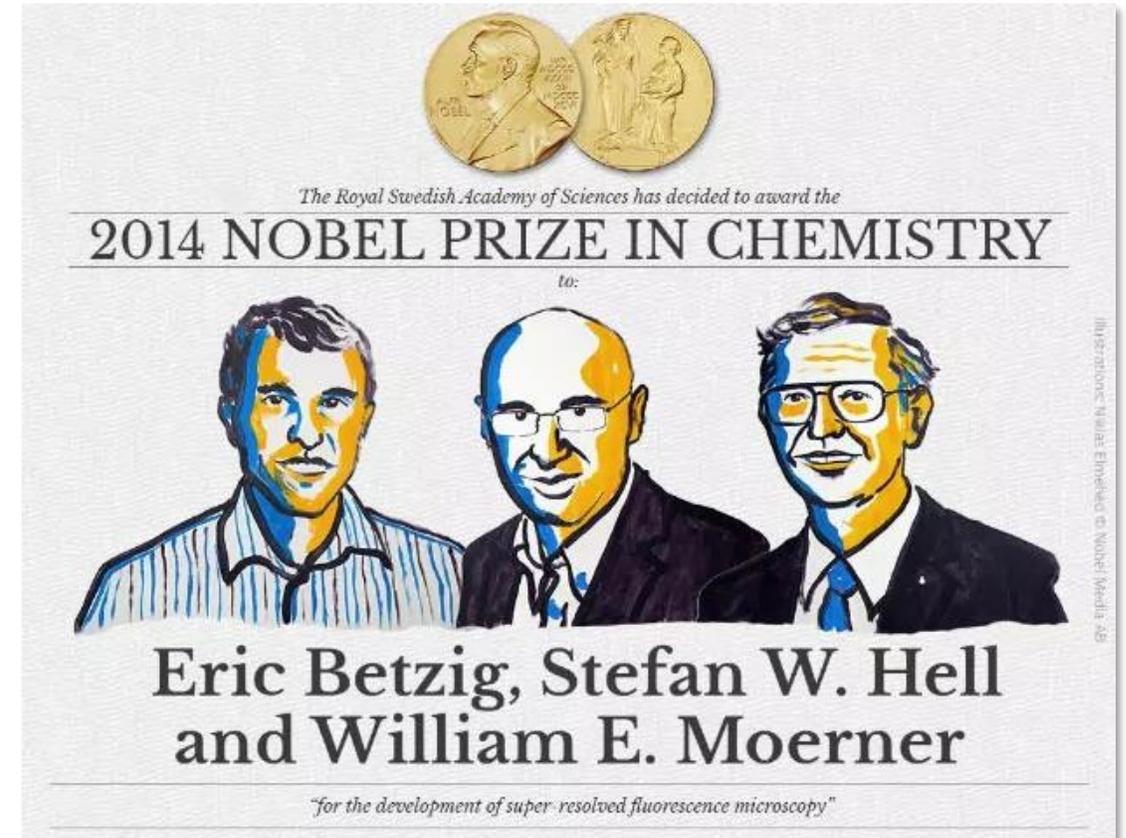
# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)

Aluminum coated SNOM tip decreases the size of the aperture and therefore increases the spatial resolution

Eric Betzig and Jay K. Trautman: “Near-field Optics: Microscopy, Spectroscopy, and Surface Modification Beyond the Diffraction limit”

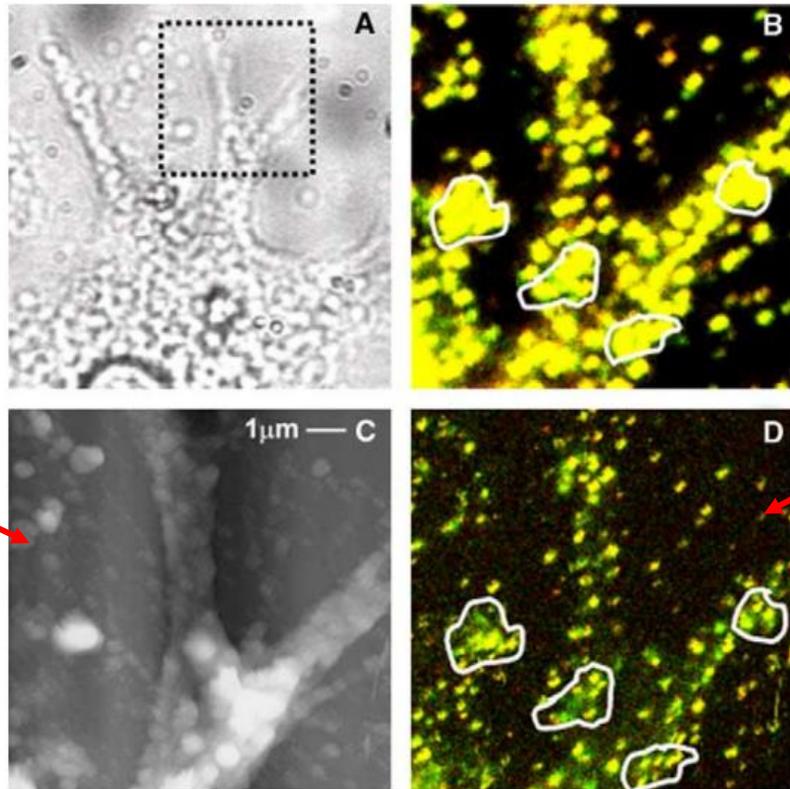


SCIENCE • VOL. 257 • 10 JULY 1992



# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)

→ **Biologia** Per la prima volta individuarono con risoluzione spaziale nanometrica molecole fluorescenti individuali su una membrana circolare in soluzione acquosa



← MICROSCOPIO OTTICO CONVENZIONALE

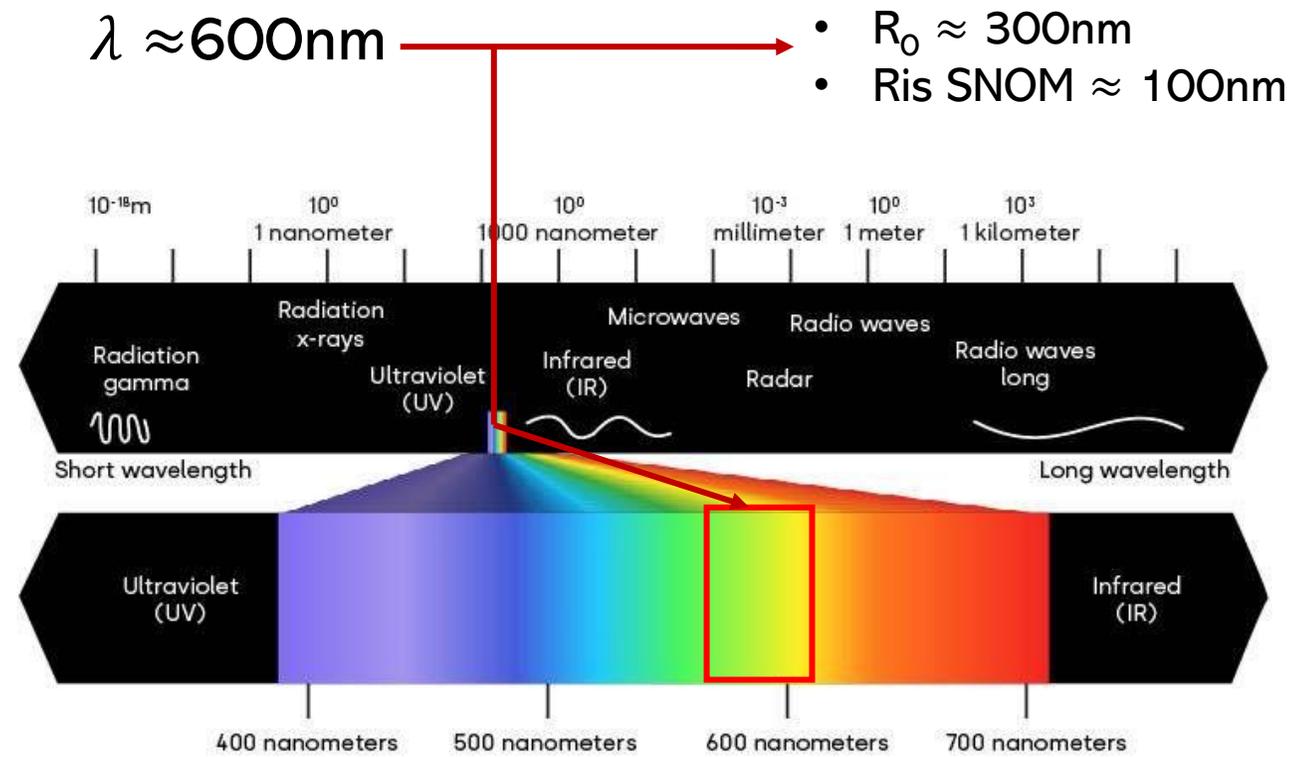
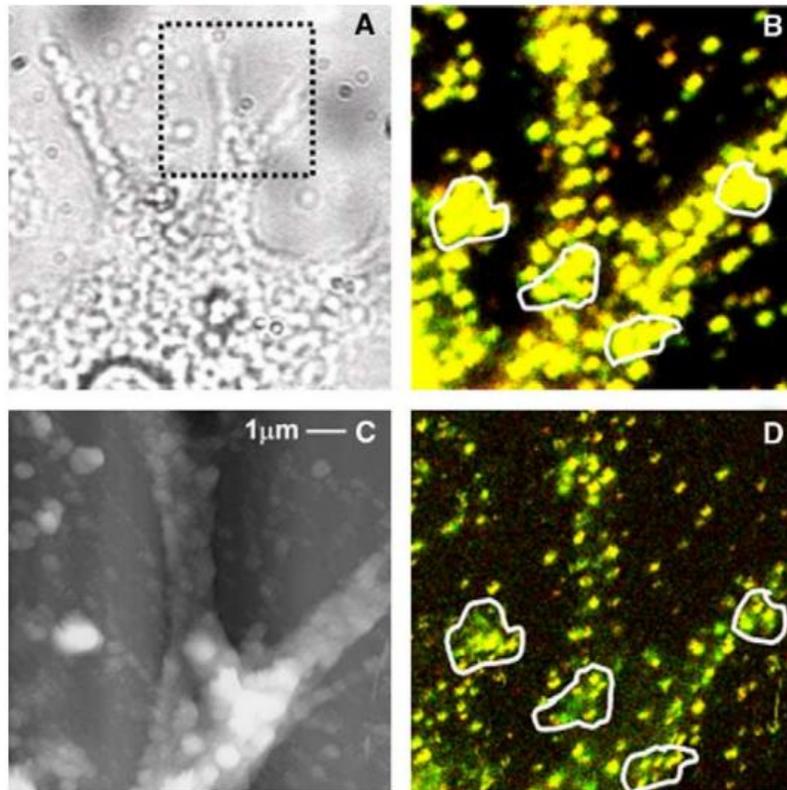
← Mappa ottica

← MICROSCOPIO SNOM

Topografia

# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)

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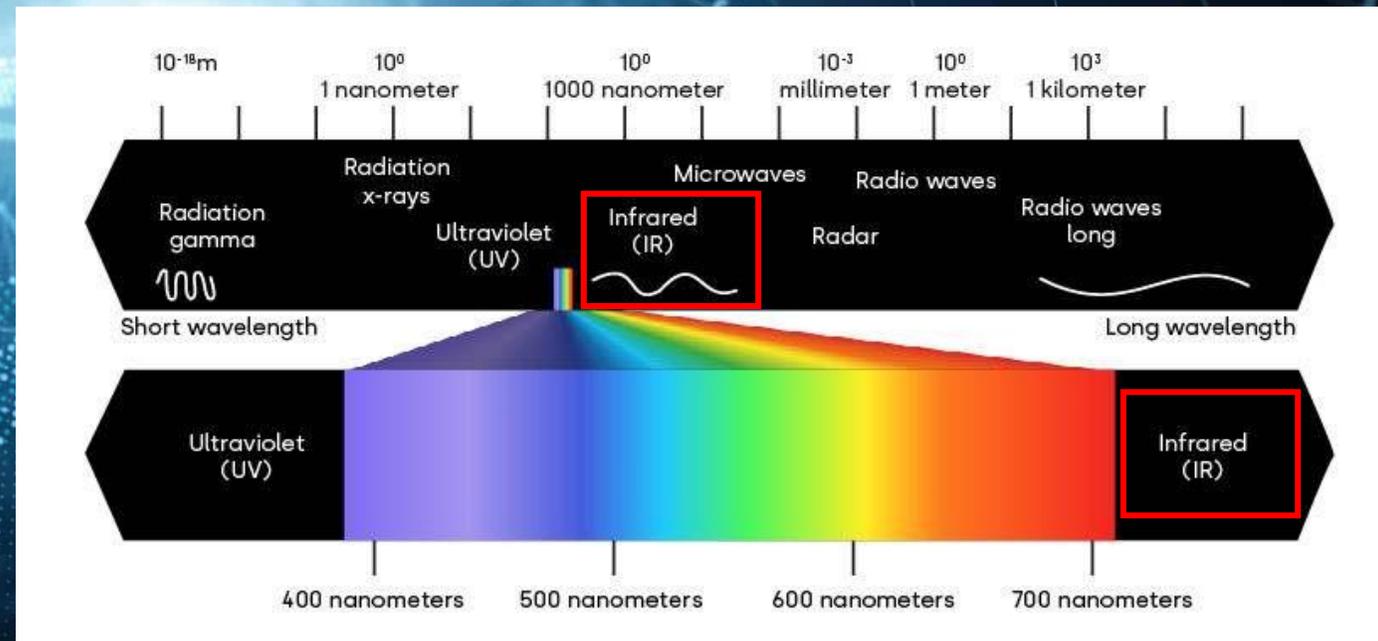


# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)

## → Telecomunicazioni

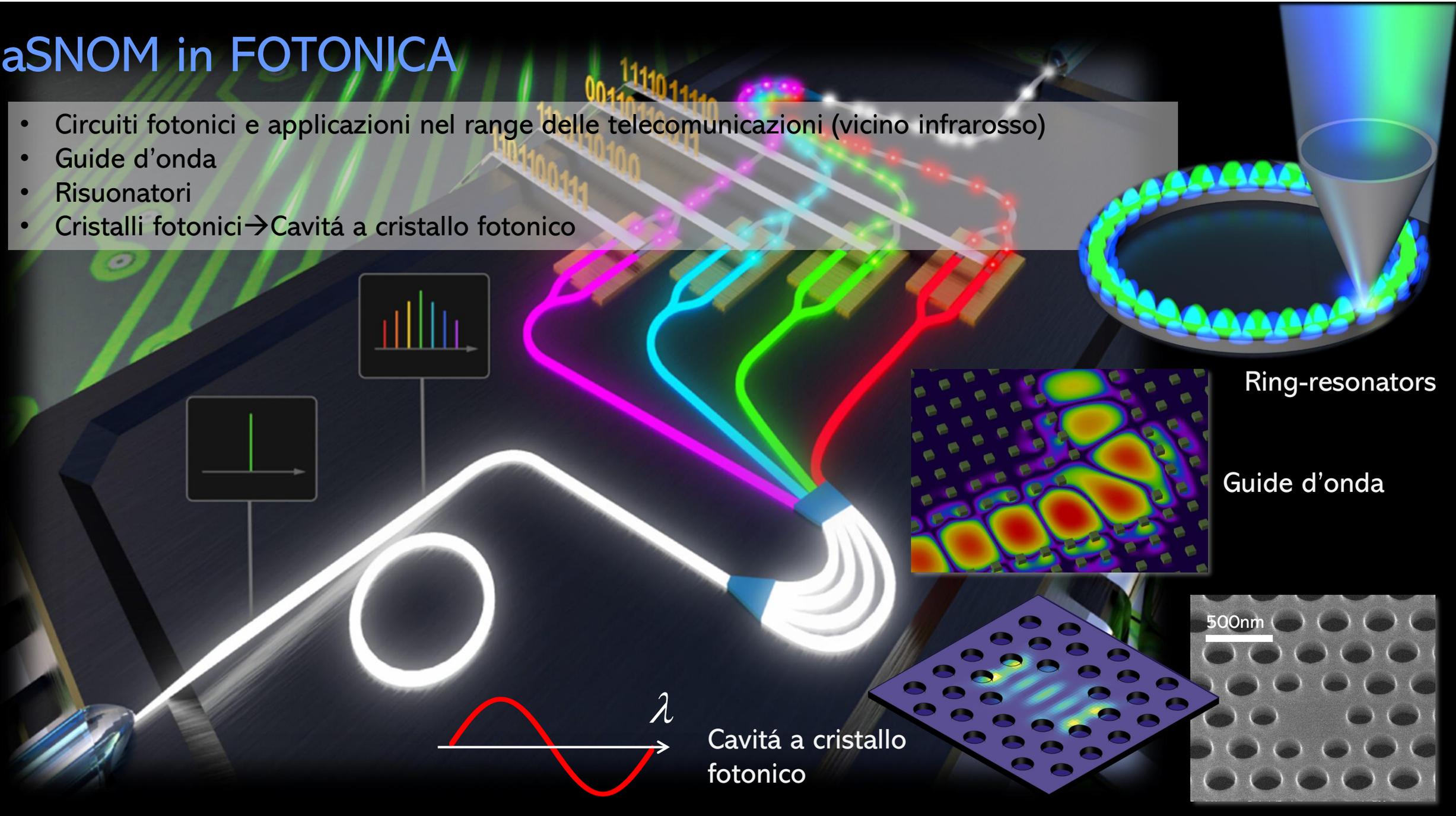
Risoluzione ottica di un microscopio peggiora ma quella dello SNOM no!

- $R_0 \approx 750\text{nm}$
- $R_{is\ SNOM} \approx \text{aperture size}$

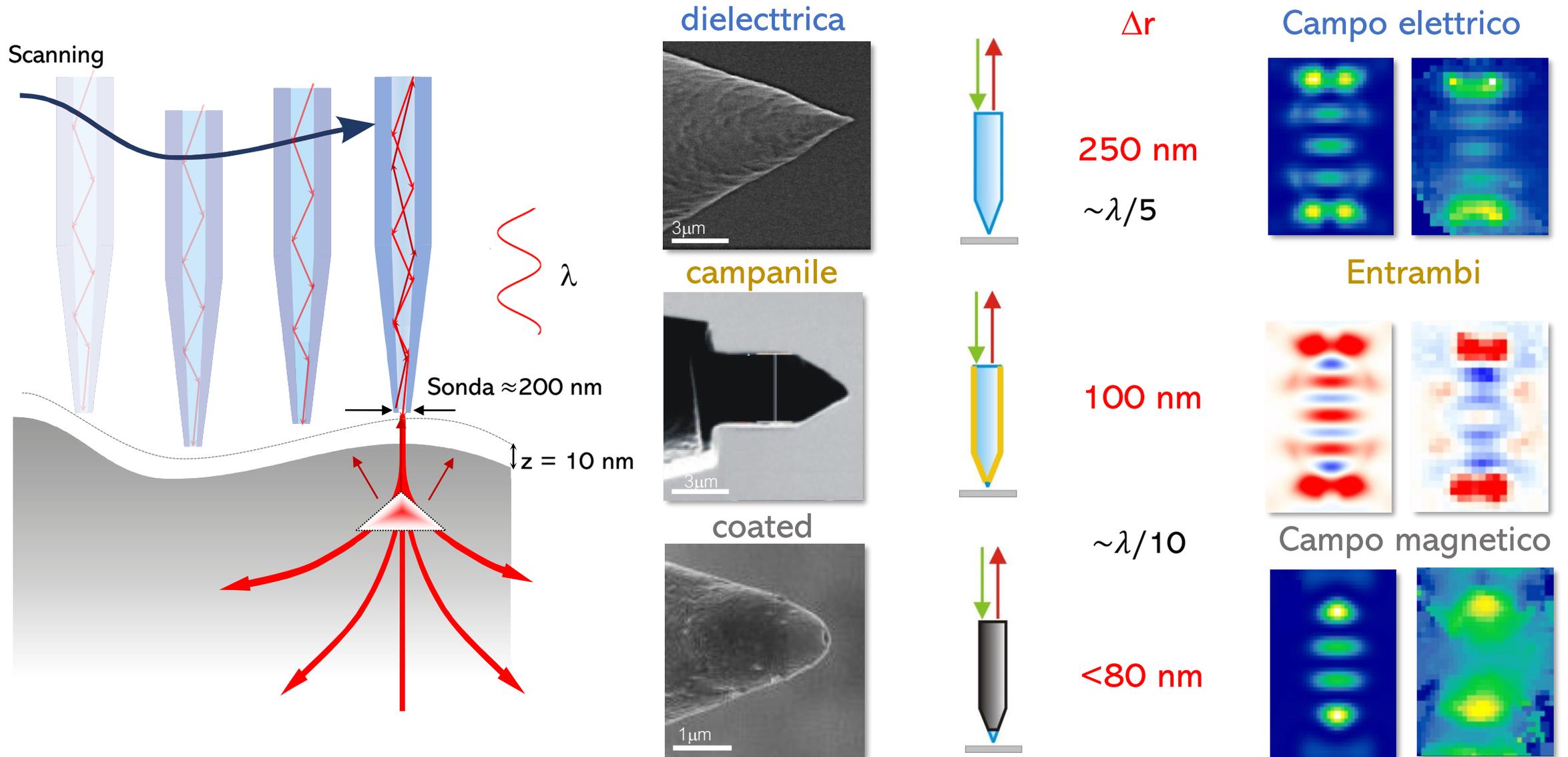


# aSNOM in FOTONICA

- Circuiti fotonici e applicazioni nel range delle telecomunicazioni (vicino infrarosso)
- Guide d'onda
- Risuonatori
- Cristalli fotonici → Cavità a cristallo fotonico



# Aperture – Scanning Near-field Optical Microscopy (a-SNOM)



# Grazie!



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