



Microscopía de Rayos X: Principios y Aplicaciones

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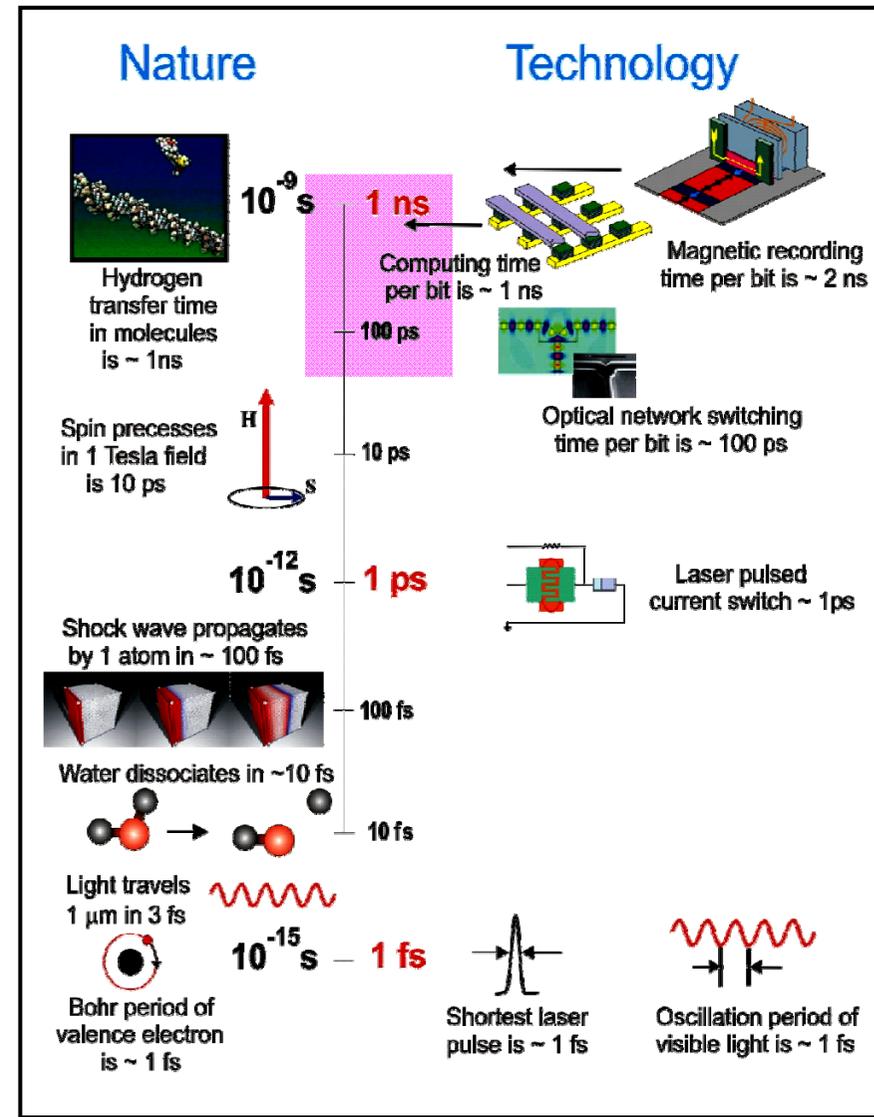
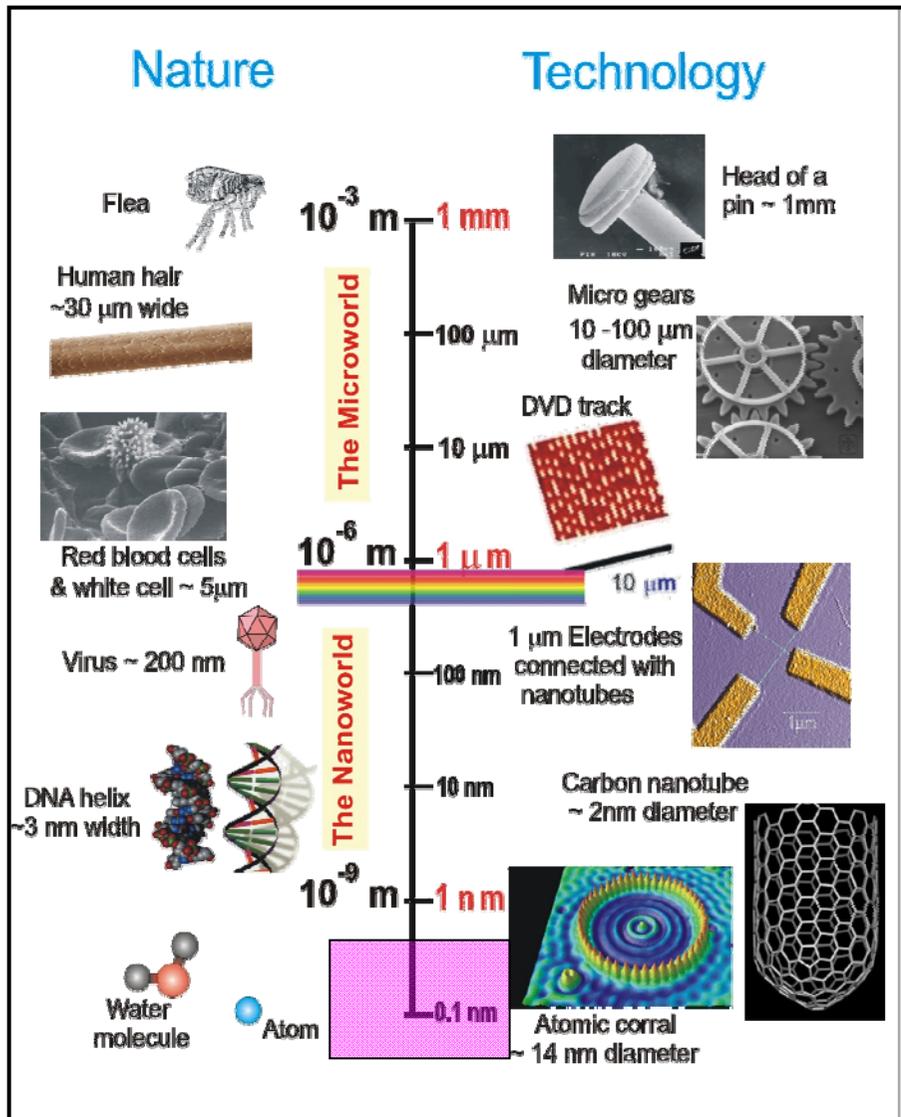
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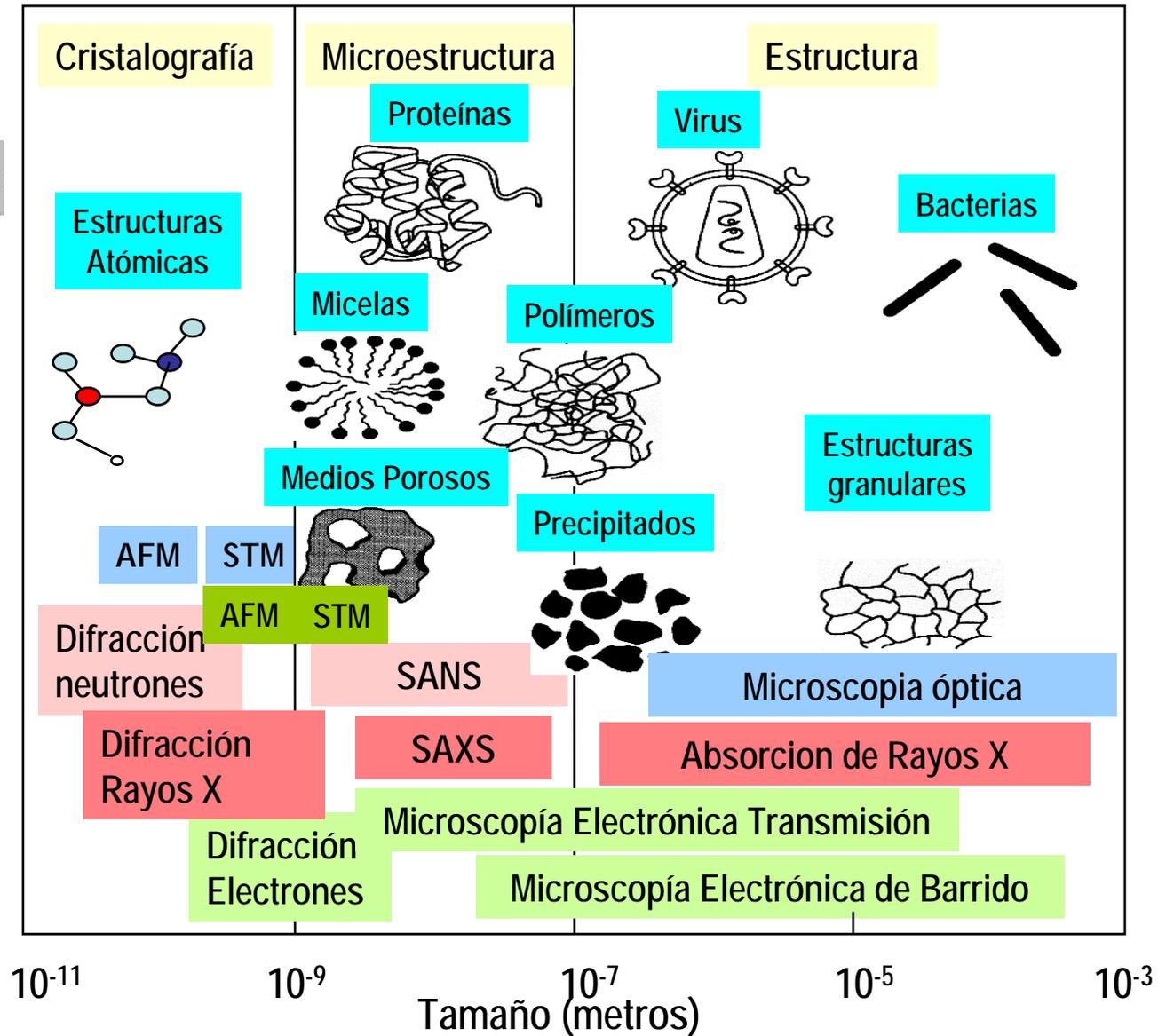
ULTRA – PEQUEÑO

ULTRA – RAPIDO

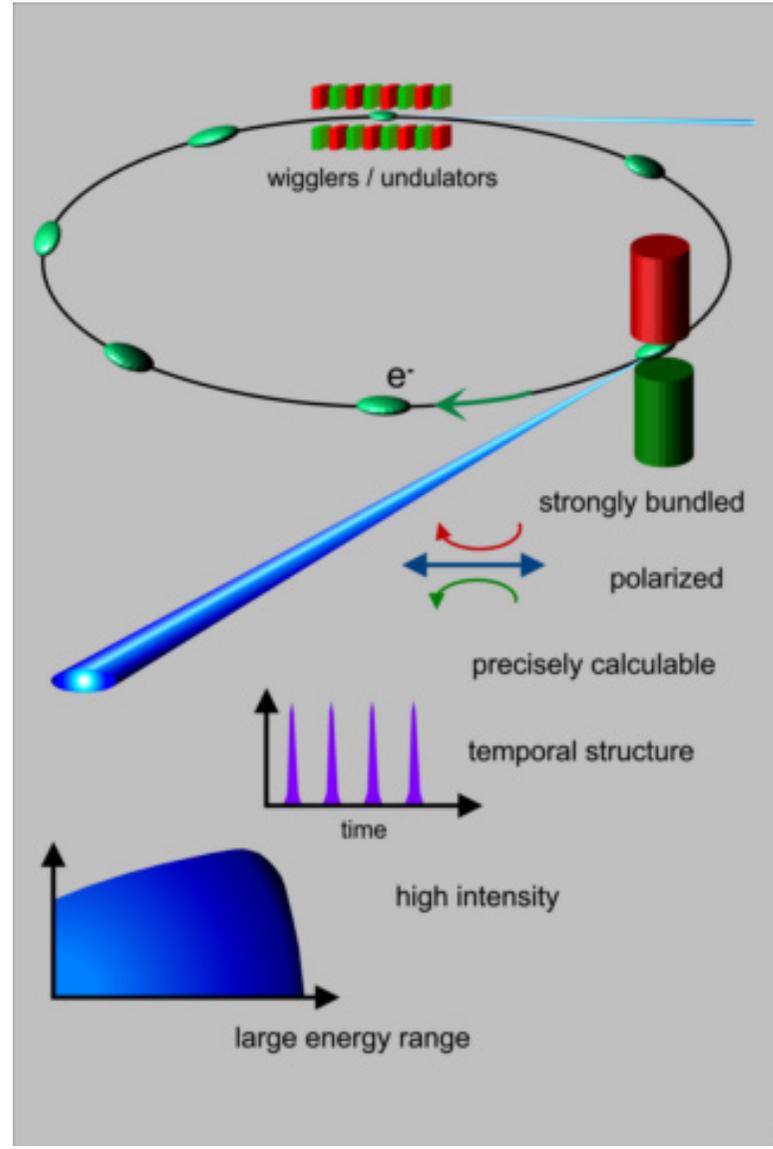
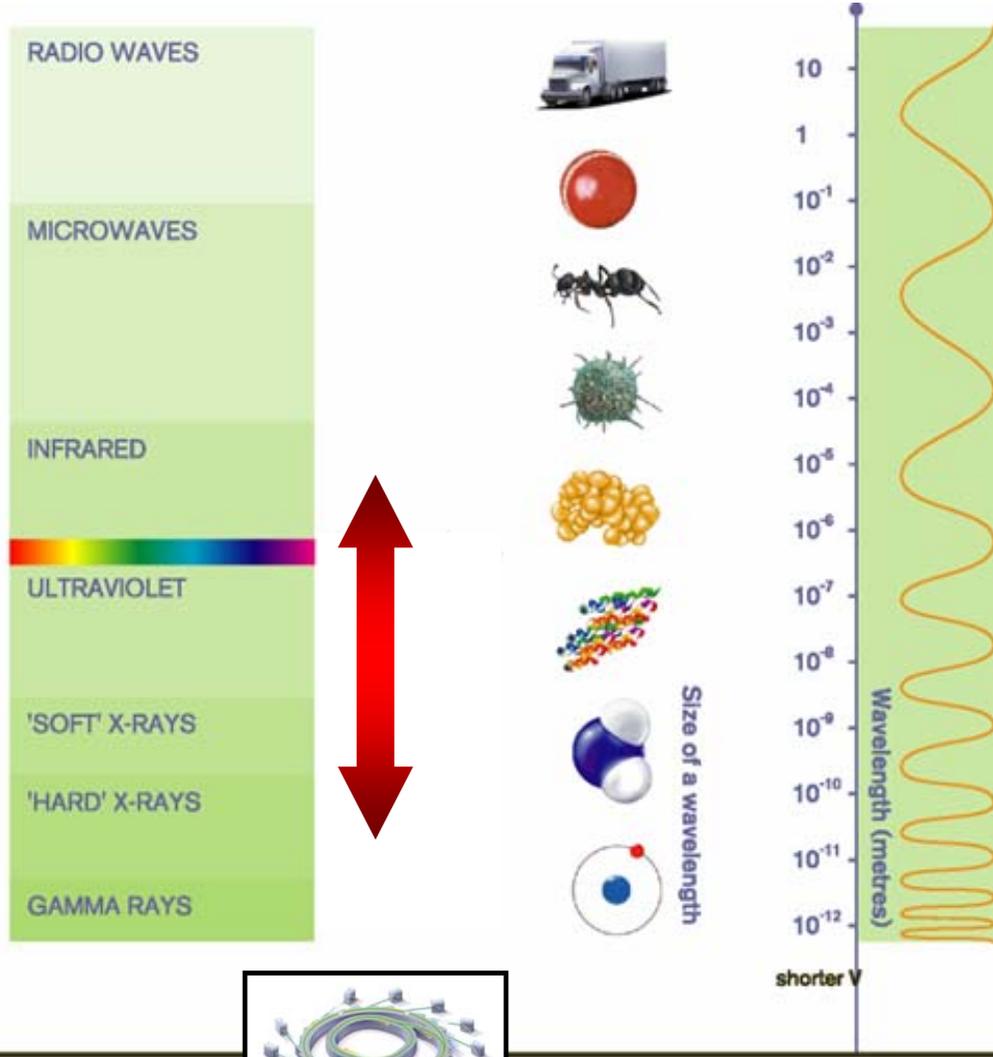


Escalas de longitud

Técnicas







Diapositiva 7

GM1

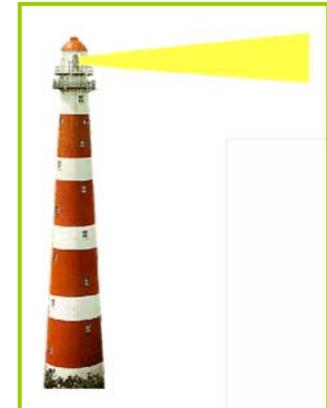
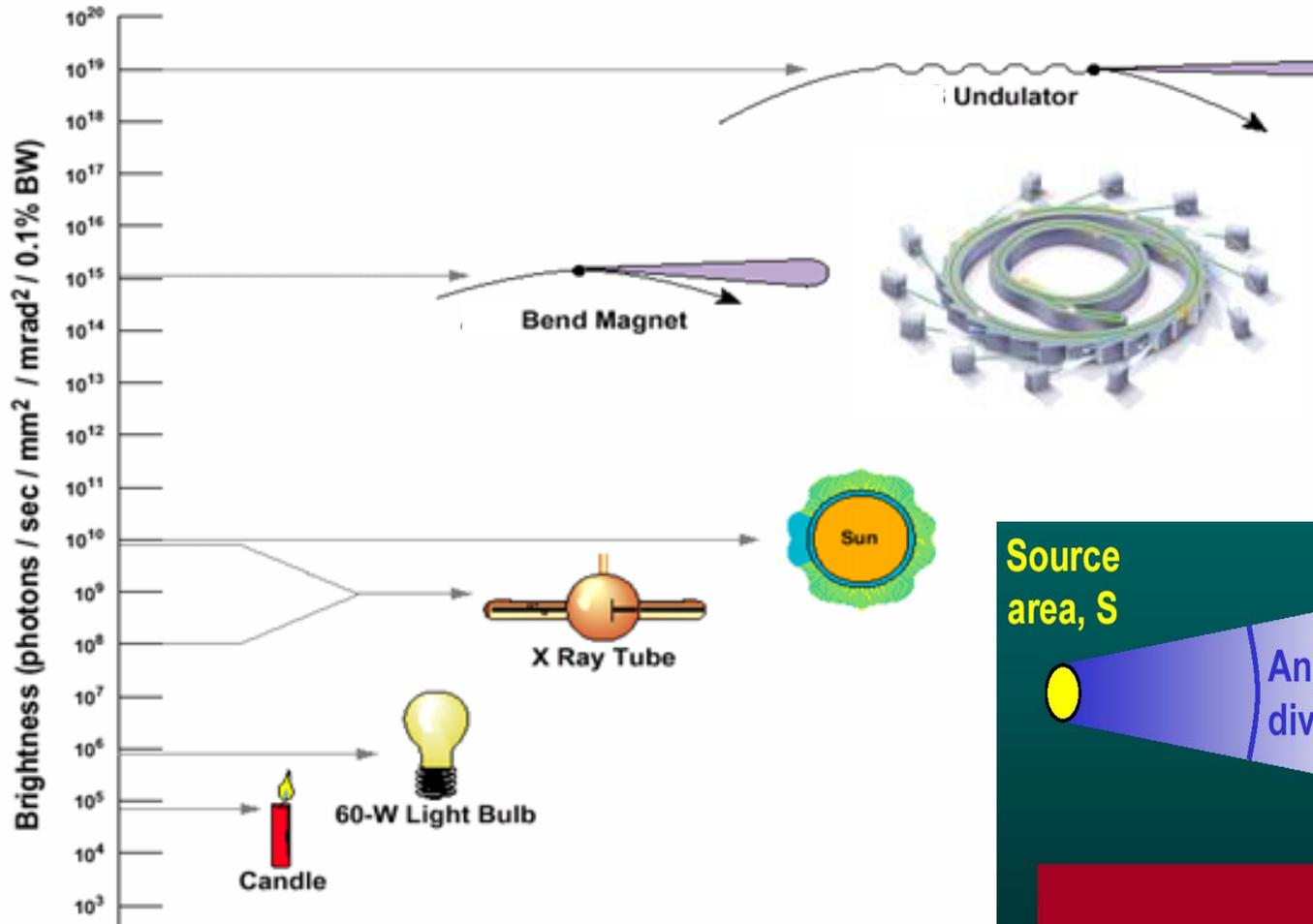
Ventajas: Muy intenso, Colimación angular, Haz coherente y polarizado, Energía sintonizable
Desventajas: Daños por radiación, Falta resolución atómica

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→ Permite comparar diferentes fuentes

mayor brillo, mayor # fotones en muestras pequeñas



Source area, S

Angular divergence, Ω

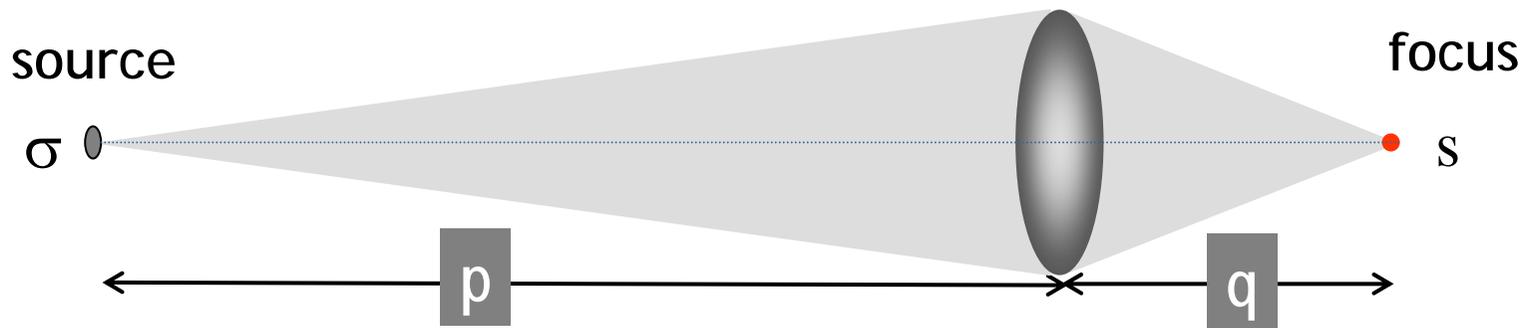
Flux, F

Brightness = constant $\times \frac{F}{S \times \Omega}$

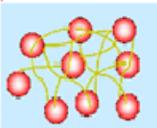
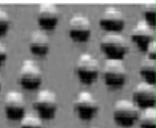
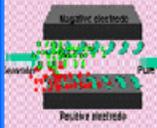
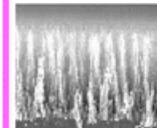
$$\text{Spectral Brightness} = \frac{\text{photon flux}}{(\Delta A) (\Delta \Omega) (\Delta \lambda / \lambda)}$$

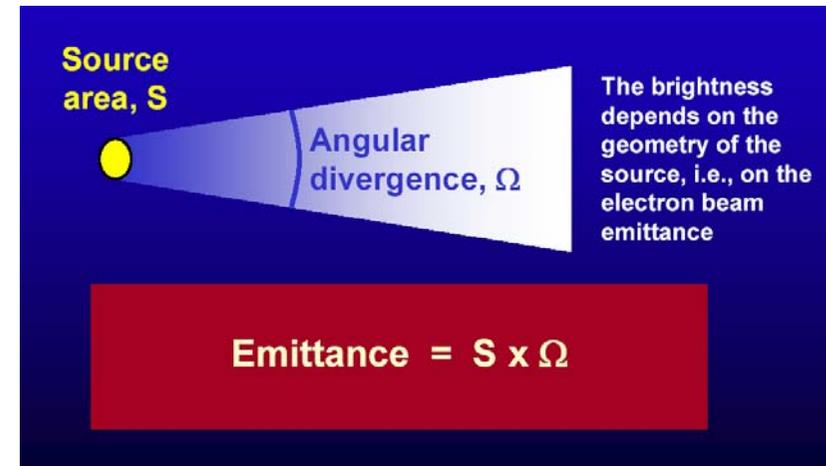
→ Permite análisis por microscopía de rayos X

mayor emitancia, mayor demagnificación



Demagnificación = p/q

Nano Materials	Bio & Medical	Electronics	Environment	Energy & others
				
Nano particle reinforcement high polymer	Drug delivery, Artificial photo-synthesis	Quantum dot, Molecular electronics	Nano structure trap, Nano filter	Hydrogen absorption & retention, Catalist, Solar batteries

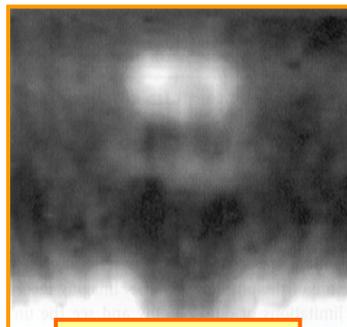
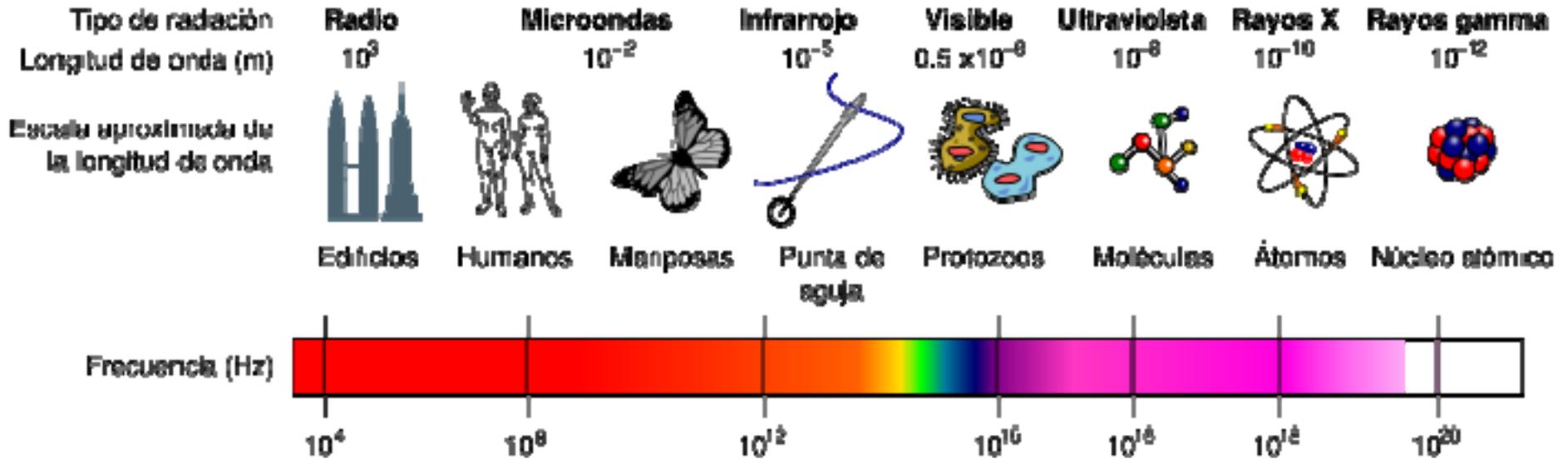


The brightness depends on the geometry of the source, i.e., on the electron beam emittance

Emittance = $S \times \Omega$

$$\epsilon_h = \sigma_h \times \sigma_h' \quad \epsilon_v = \sigma_v \times \sigma_v'$$

Diffraction limited



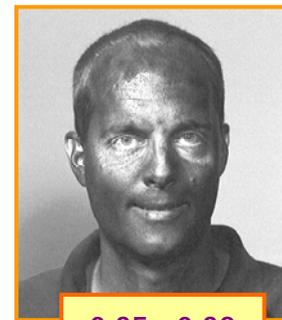
3.3 mm



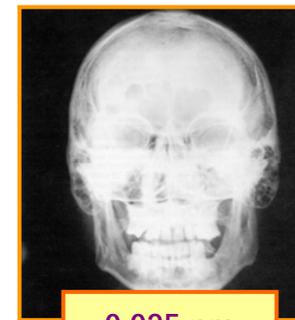
8 - 9 μm
(infrarrojo)



0.4 - 0.7 μm
(visible)



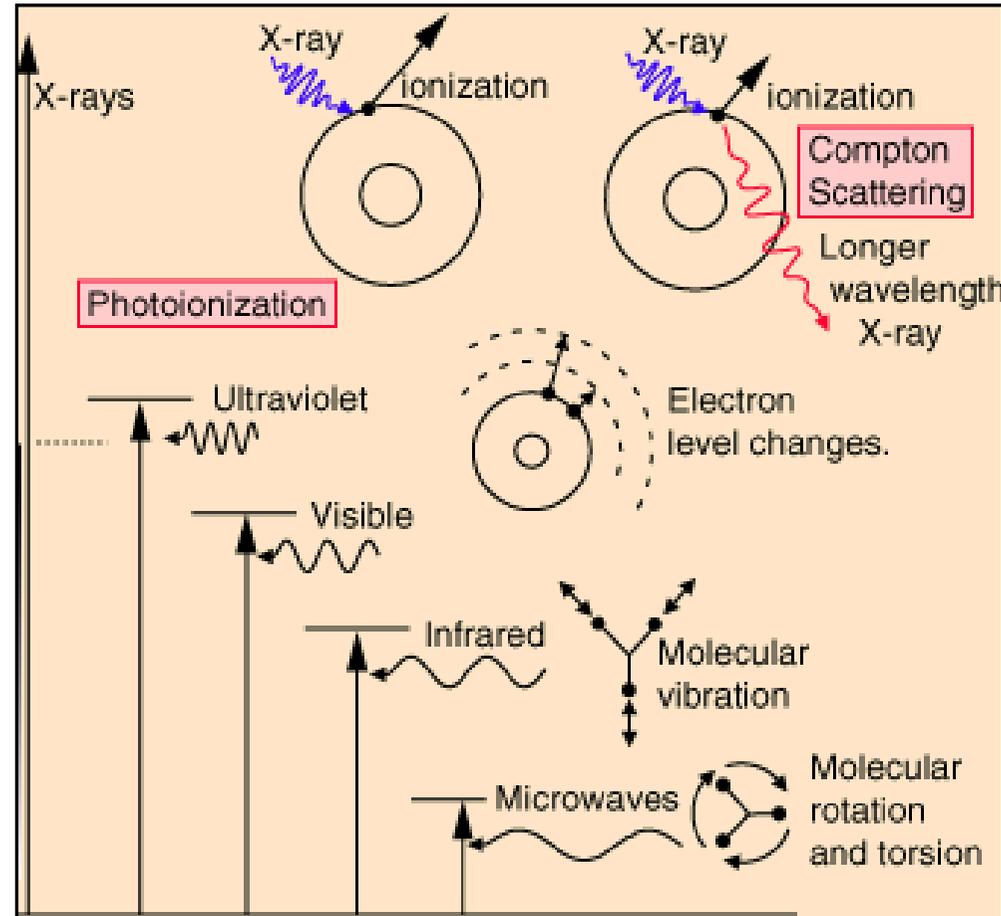
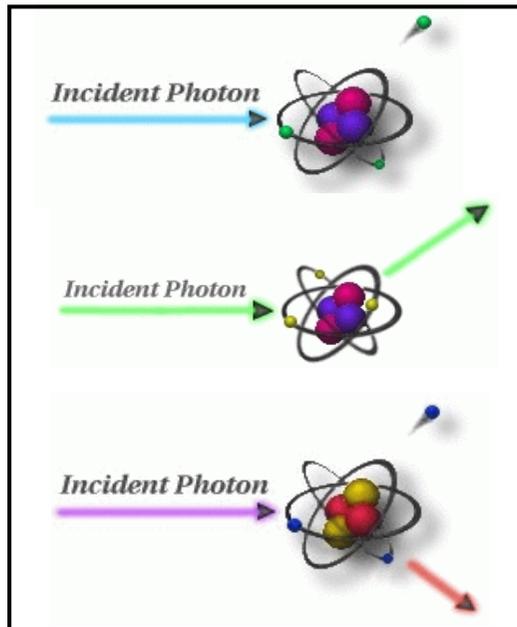
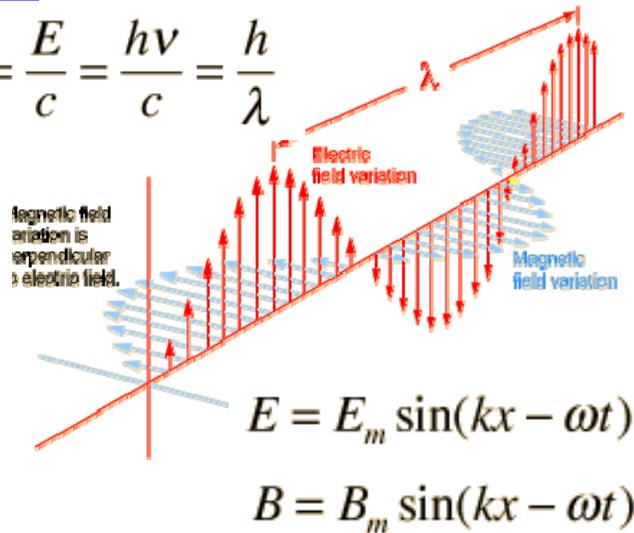
0.35 - 0.38 μm
(UV)



0.025 nm
(rayos X)



$$p = \frac{E}{c} = \frac{h\nu}{c} = \frac{h}{\lambda}$$



Sección eficaz total

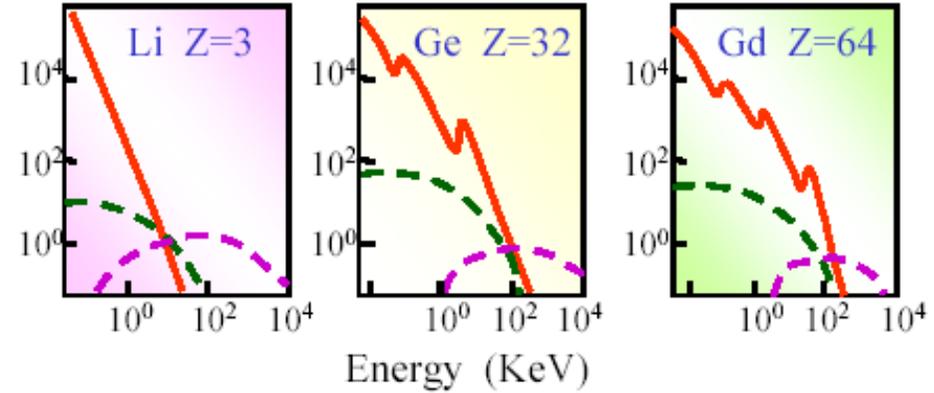
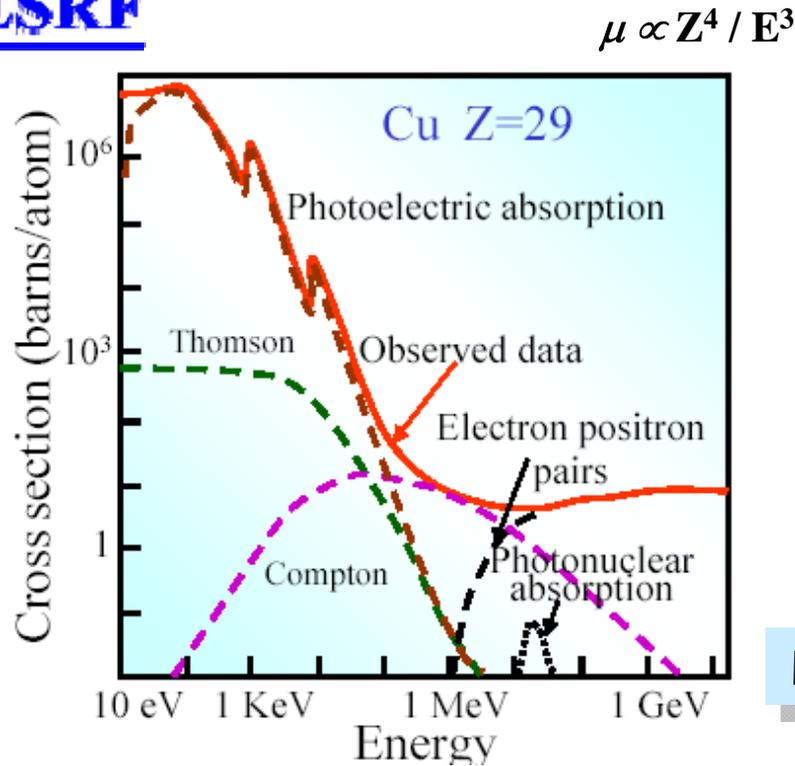
$$\sigma_{tot} = \sigma_{coh} + \sigma_{incoh} + \sigma_{ph} + \tau + \kappa_e + \kappa_n$$

Diapositiva 11

GM2

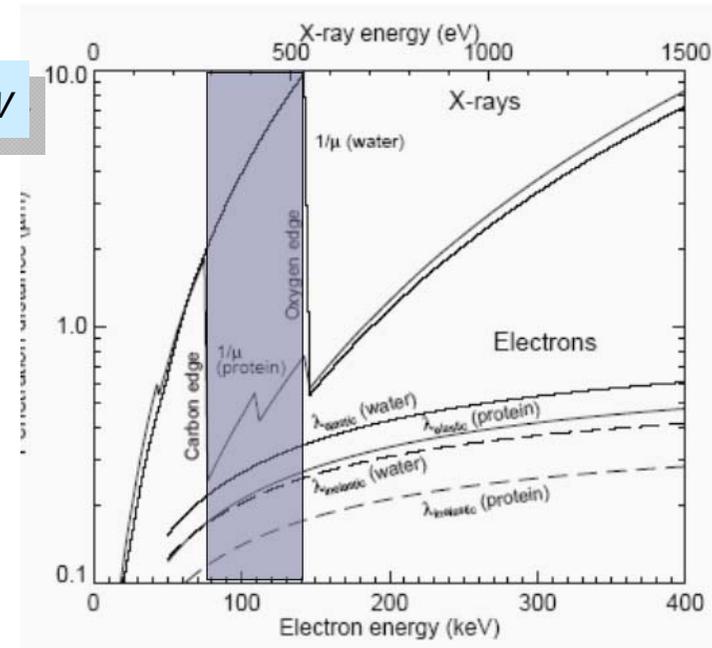
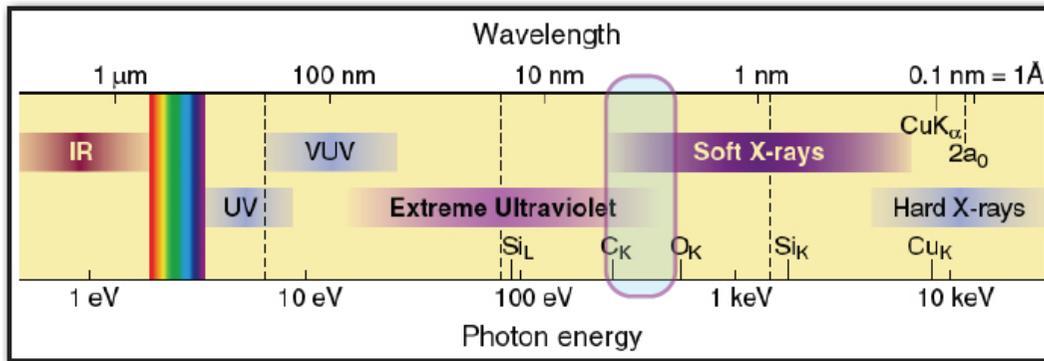
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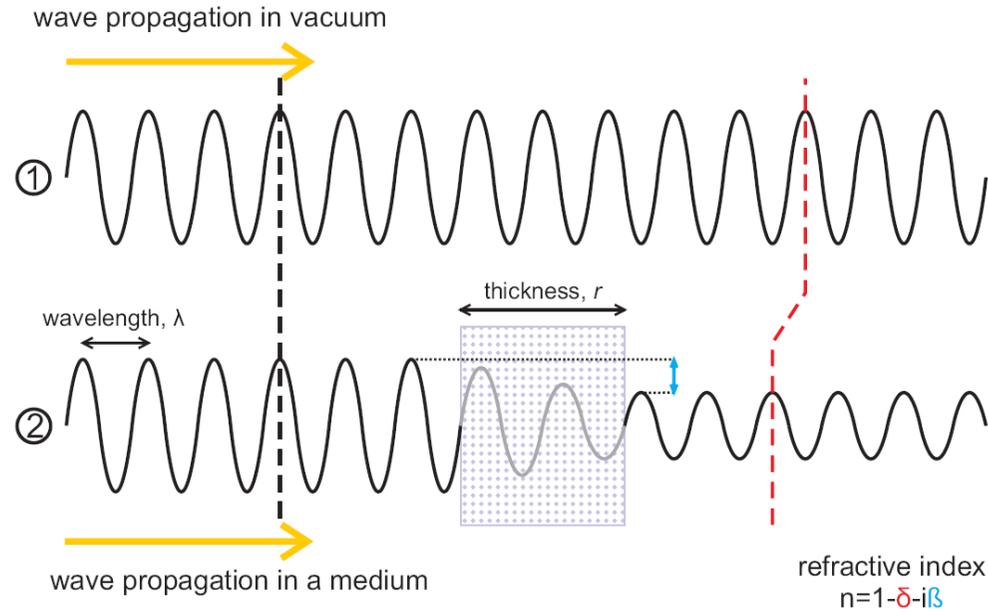
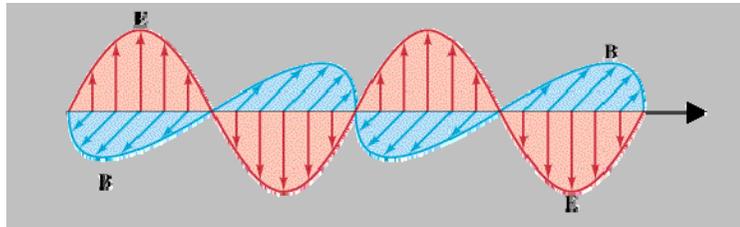


$$\sigma_a = \sigma_{tot} = \sigma_{incoh} + \sigma_{coh} + \tau$$

Water Window



$$\mathbf{E} = \mathbf{E}_0 e^{-i(\omega t - \mathbf{k}\mathbf{r})} \quad |\mathbf{k}| = k = \frac{\omega}{c} n \quad n = 1 - \delta + i\beta$$



$$\left\{ \begin{array}{l} \delta = \frac{n_a r_e \lambda^2}{2\pi} f_1^0(\omega) \\ \beta = \frac{n_a r_e \lambda^2}{2\pi} f_2^0(\omega) \\ \mu = (4\pi/\lambda)\beta \\ \eta = (2\pi/\lambda)\delta \end{array} \right.$$

$$\mathbf{E}(\mathbf{r}, t) = \mathbf{E}_0 \underbrace{e^{i\omega(t - \frac{r}{c})}}_{\text{vacuum propagation}} \underbrace{e^{-i(2\pi \frac{\delta}{\lambda})r}}_{\phi\text{-shift}} \underbrace{e^{-(2\pi \frac{\beta}{\lambda})r}}_{\text{absorption decay}}$$

$$\delta \sim 10^{-5} \Rightarrow n \sim 10^{-5}$$

$$n < 1$$

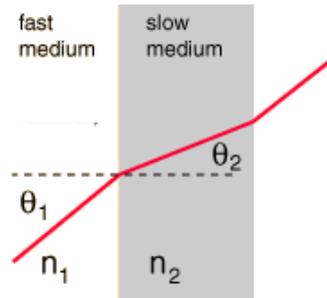
Mecanismos de contraste: absorción / fase



Luz visible

$$\frac{n_1}{n_2} = \frac{\sin \theta_2}{\sin \theta_1}$$

Ley de Snell



Rayos X

$$n(\omega) = 1 - \delta(\omega) + i\beta(\omega)$$

$$n < 1$$

$$v_p = \frac{c}{n} > c \quad v_p = \frac{\omega}{k} = \left(\frac{\gamma - 1}{\gamma \beta^2} \right) v_g$$

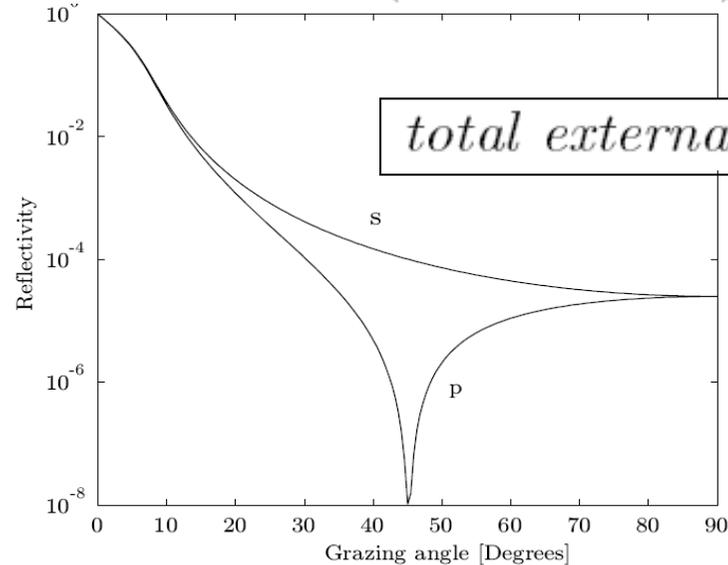
Velocidad de fase en el medio > c

$$\theta_1 = \theta_c \rightarrow \theta_2 = 90^\circ$$

low reflectivity except at grazing angles (i.e., θ small)

$$R_s = \frac{|n_1 \cos \phi_1 - n_2 \cos \phi_2|^2}{|n_1 \cos \phi_1 + n_2 \cos \phi_2|^2}$$

$$R_p = \frac{|n_2 \cos \phi_1 - n_1 \cos \phi_2|^2}{|n_2 \cos \phi_1 + n_1 \cos \phi_2|^2}$$



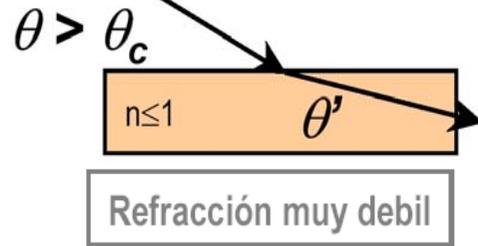
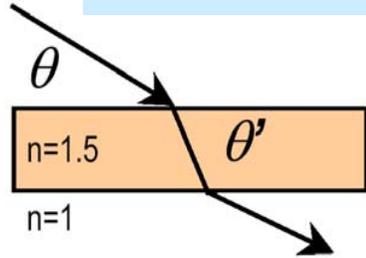
$$\theta_c = \sqrt{2\delta}$$

$$\theta_c \sim \text{mrad}$$

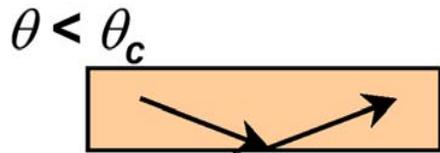
Focalización en superficies curvas

Luz visible

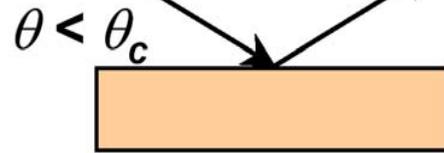
Rayos X



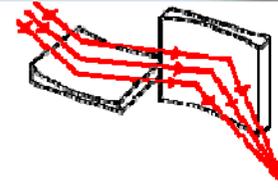
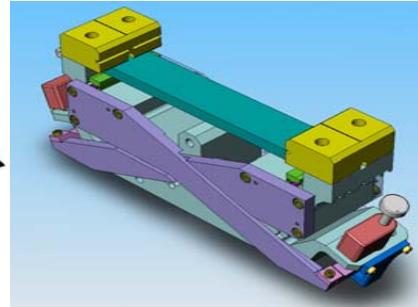
Refracción muy debil



Reflexión total interna

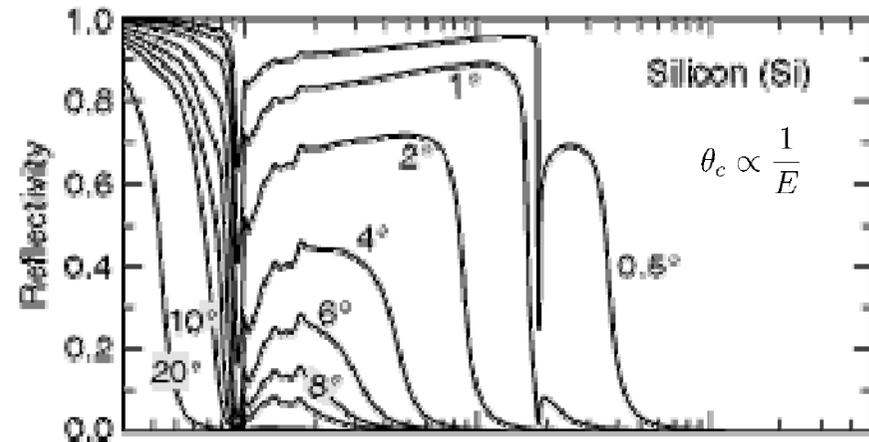
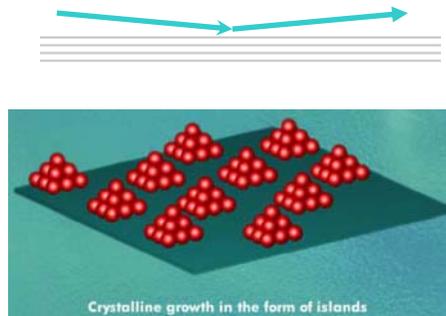
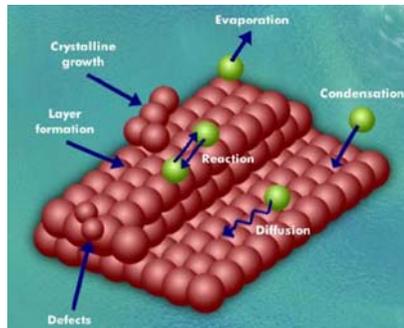


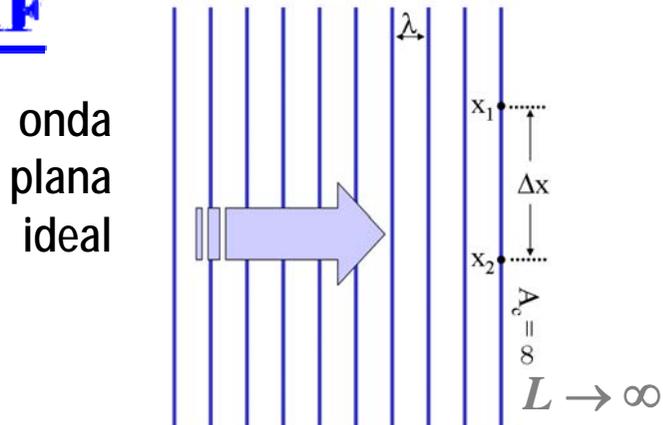
Reflexión total externa



Filtro energético

Sensibilidad superficial



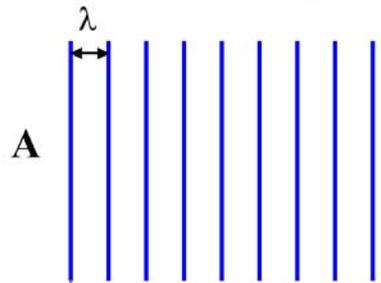


HAZ REAL

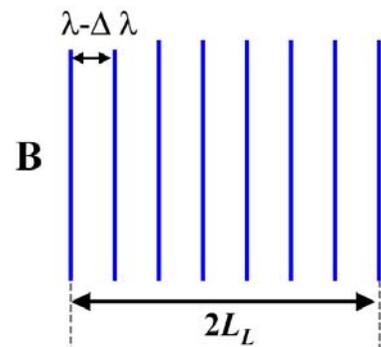
no es perfectamente monocromático

no se propaga en una dirección perfectamente definida

→ HAZ NO ES EXACTAMENTE MONOCROMÁTICO



→ $2L_L = N \lambda$



→ $2L_L = (N+1) (\lambda - \Delta \lambda)$

L_L Longitud de coherencia longitudinal:
ondas en contra-fase

$(N+1) (\Delta \lambda) = \lambda \rightarrow N \approx \lambda / \Delta \lambda$

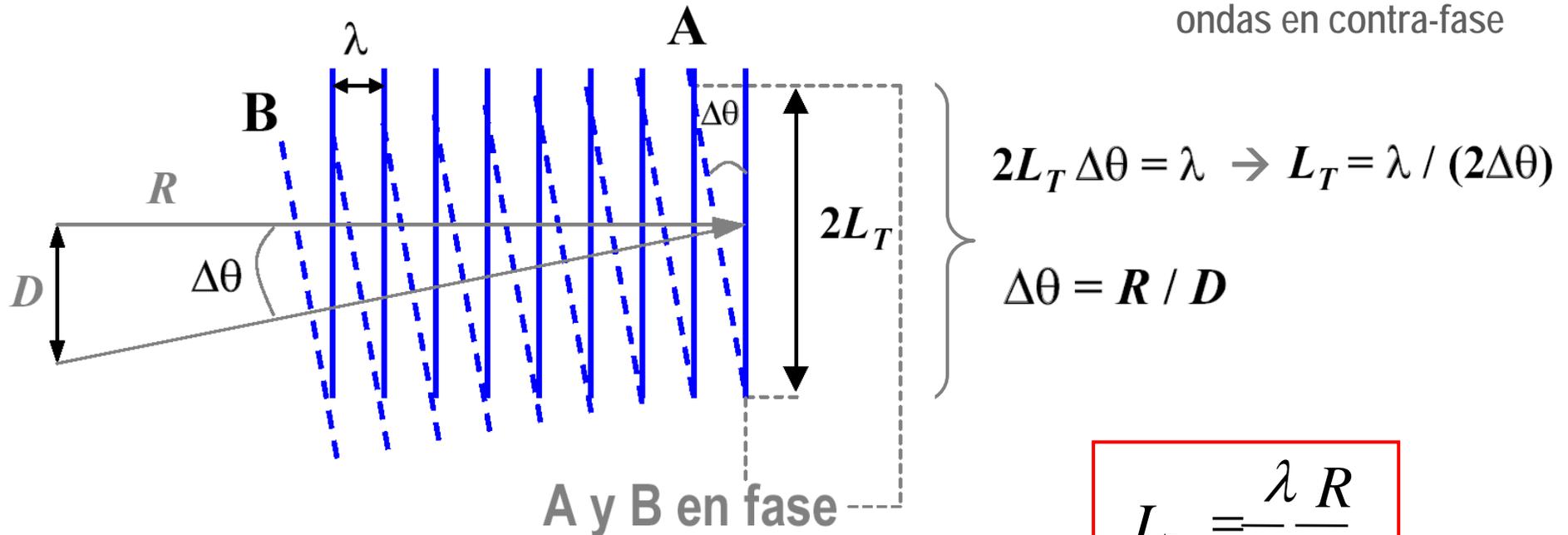
$$L_L = \frac{\lambda}{2} \frac{\lambda}{\Delta \lambda}$$

--- A y B en fase ---

Ejemplo: $E=10 \text{ keV}$, $\lambda=1.2398 \text{ \AA}$, $\Delta \lambda/\lambda=10^{-4} \rightarrow L_L=6170 \text{ \AA} = 0.6 \text{ \mu m}$

→ HAZ NO SE PROPAGA EXACTAMENTE EN UNA DIRECCIÓN BIEN DEFINIDA

L_T Longitud de coherencia transversal:
ondas en contra-fase



$$L_T = \frac{\lambda R}{2 D}$$

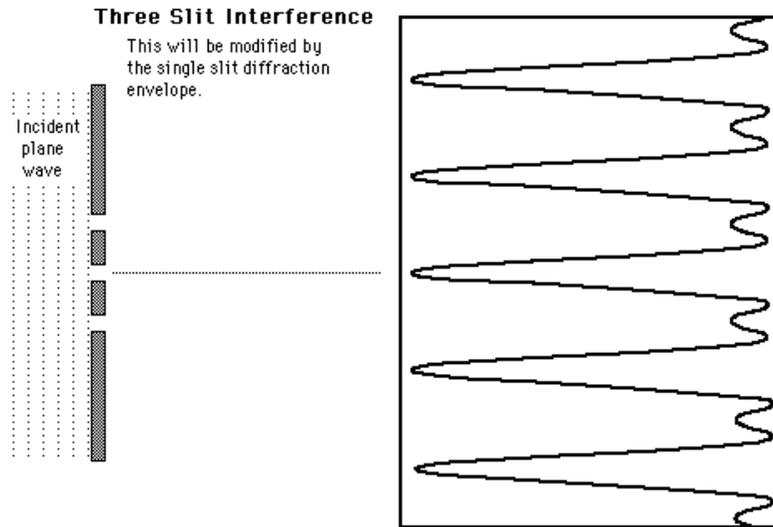
R : distancia fuente – muestra

D : tamaño de la fuente

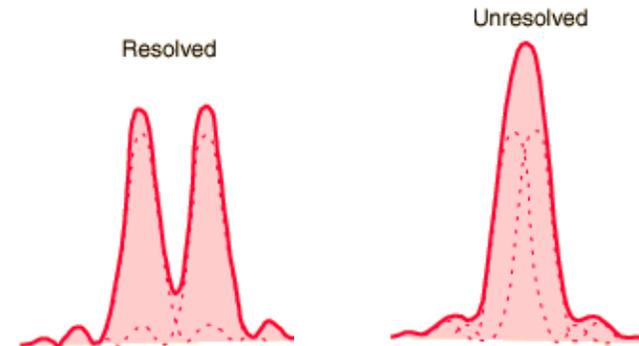
Ejemplo: $E=10$ keV, $\lambda=1.2398$ Å, $D=30$ μm, $R=30$ m, $\Delta\lambda/\lambda=10^{-4}$

→ $L_T = 62$ μm

Longitud de coherencia finita

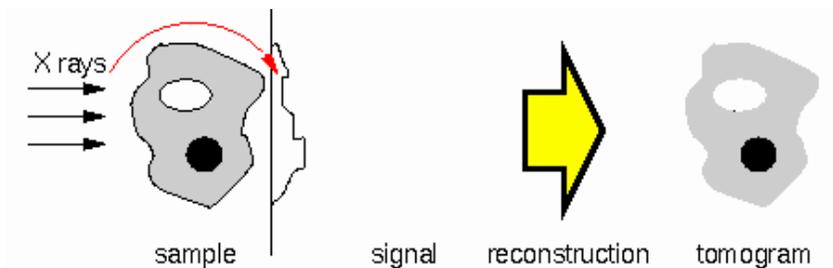


Interferencia: limite de separacion de dos objetos

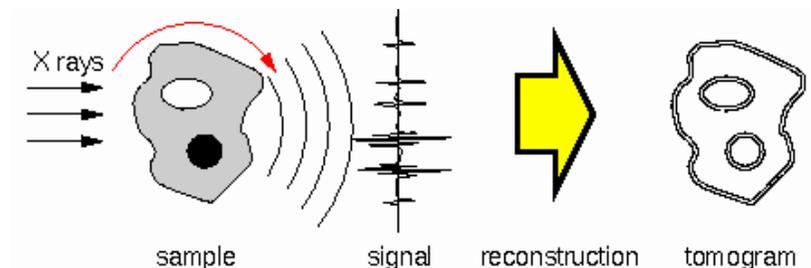


Formación de Imágenes

conventional absorption imaging



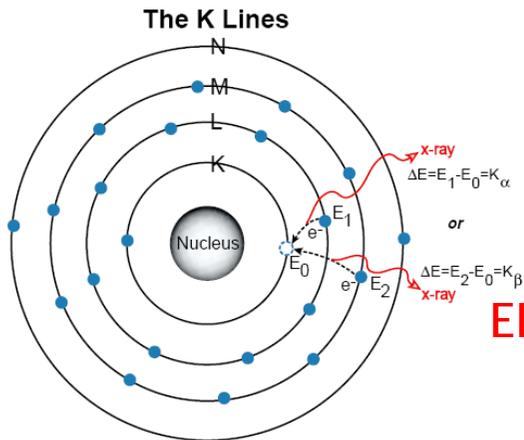
phase - contrast imaging



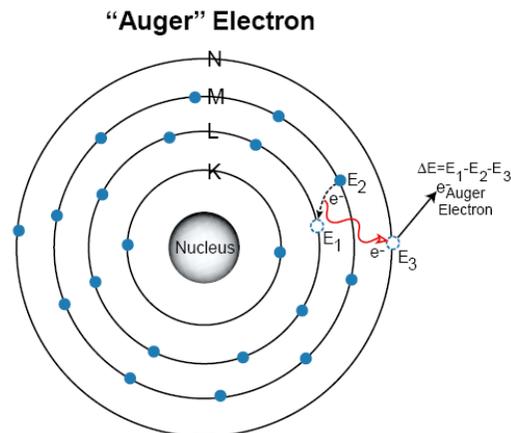
poca absorción, pero diferentes índices de refracción

- Energía de excitación excede la energía de ionización
- Procesos de relajación →

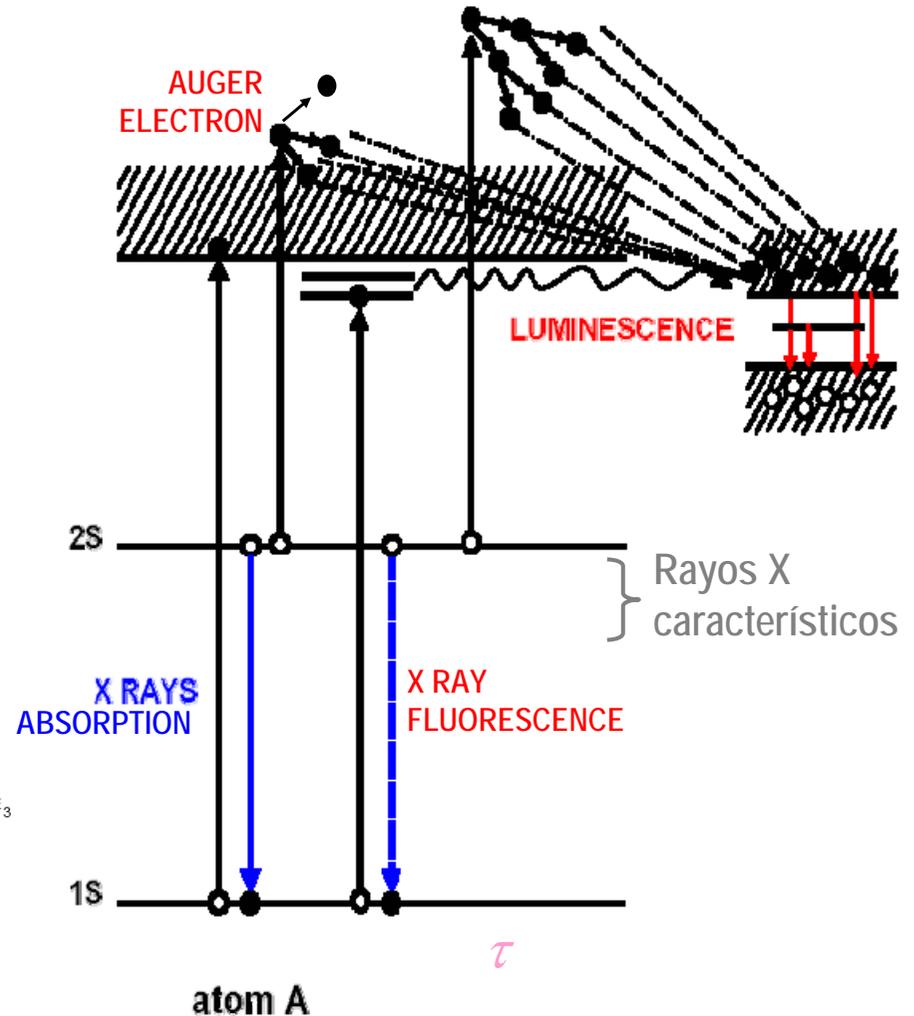
FLUORESCENCIA DE RAYOS X



ELECTRONES AUGER

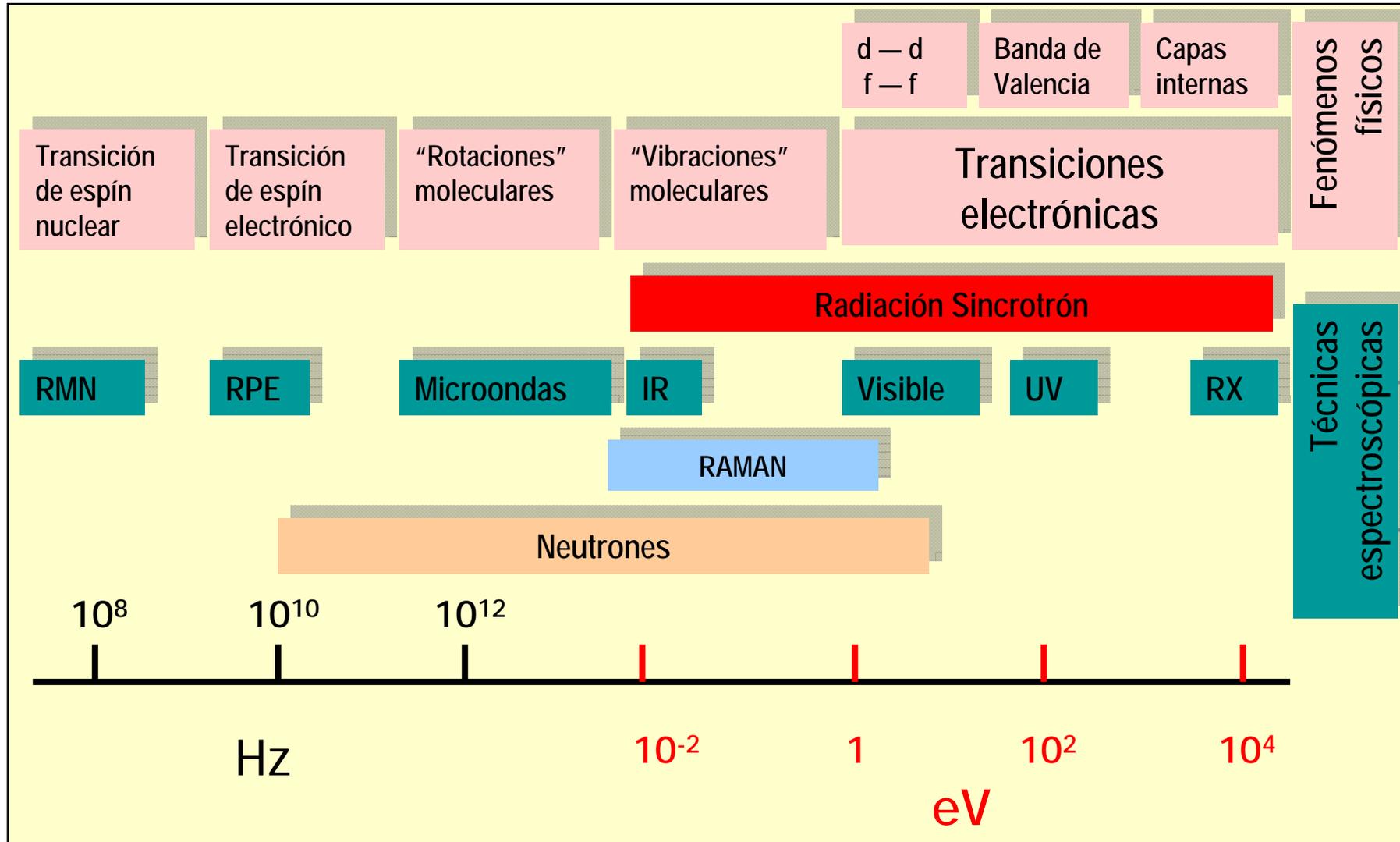


ABSORCIÓN FOTOELÉCTRICA





Mecanismos de contraste: XRF, XAS, XRD, XPEEM, XLD, XEOL, ...





Indice

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- Aplicaciones

La emitancia del haz de electrones es una constante a lo largo de su órbita

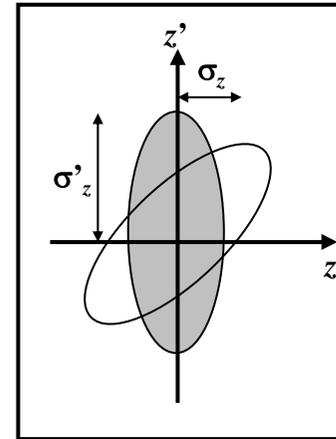
$$\varepsilon_z = \sigma_z \sigma'_z = \text{cte}$$

- Focalización del haz → Menor tamaño del haz, mayor divergencia
- Colimación → Menor divergencia del haz, mayor tamaño

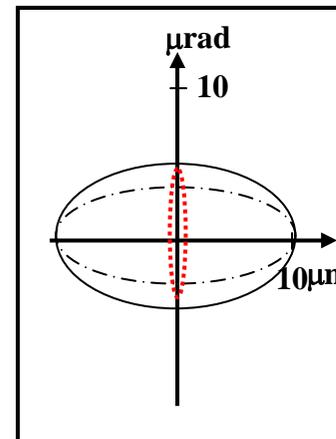


Debido a la absorción y la eficiencia de los elementos de la línea de luz, la densidad de fotones en el espacio de fase disminuye

Espacio de fase



Haz de electrones en z



Haz de fotones en z

Área elipse constante

Valores típicos (v):

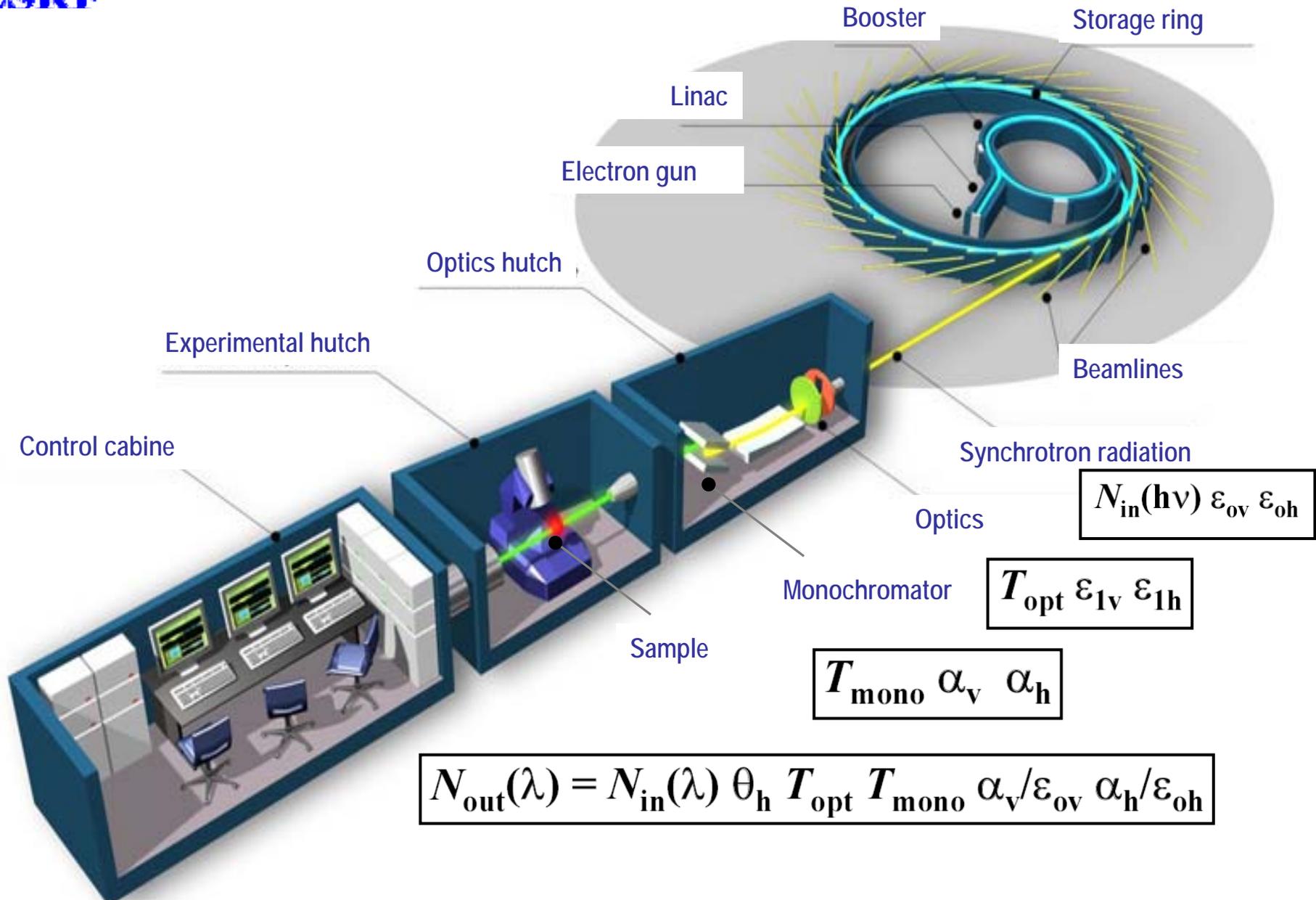
$$\sigma_z = 10.3 \mu\text{m}$$

$$\sigma'_z = 3.8 \mu\text{rad}$$



$$\varepsilon_z = \sigma \sigma'_z = 39 \text{ pm rad}$$

- electrones
- fotones
- · Fotones (diffraction limit)

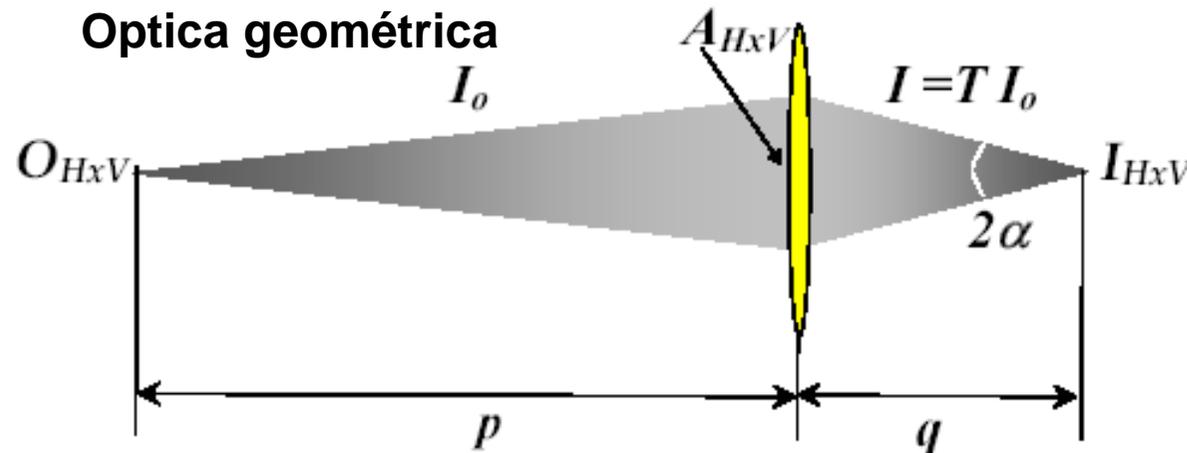


$$N_{in}(h\nu) \epsilon_{ov} \epsilon_{oh}$$

$$T_{opt} \epsilon_{1v} \epsilon_{1h}$$

$$T_{mono} \alpha_v \alpha_h$$

$$N_{out}(\lambda) = N_{in}(\lambda) \theta_h T_{opt} T_{mono} \alpha_v/\epsilon_{ov} \alpha_h/\epsilon_{oh}$$



Focal length $\frac{1}{p} + \frac{1}{q} = \frac{1}{f}$

Demagnification $M = q/p$

Numerical Aperture $NA = \sin \alpha$

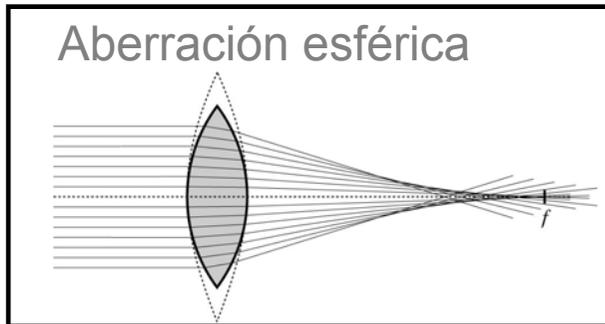
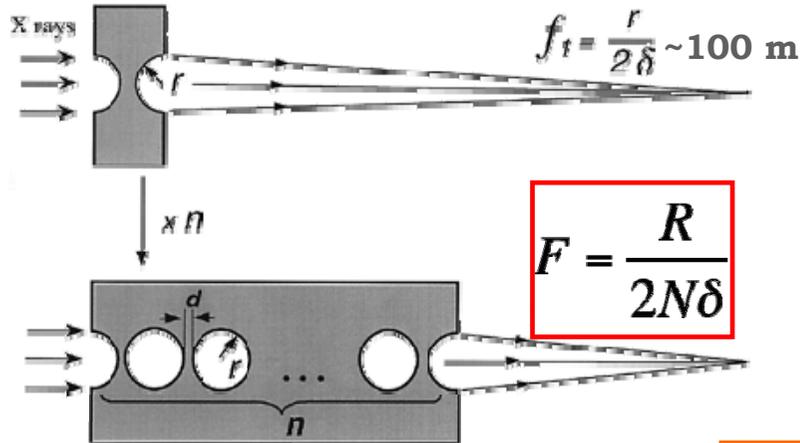
Gain $G = T A_{HxV} / I_{HxV}$

Resolution $\Delta = 0,66\lambda / NA$

Ej:
 $G = 10^3 - 10^4$
 $M = 100$
 $T = 20\%$
 $A = 100 \mu m^2$

Cuando $|q| \gg |p|$, a p se le llama la longitud focal de la lente o longitud focal objeto f

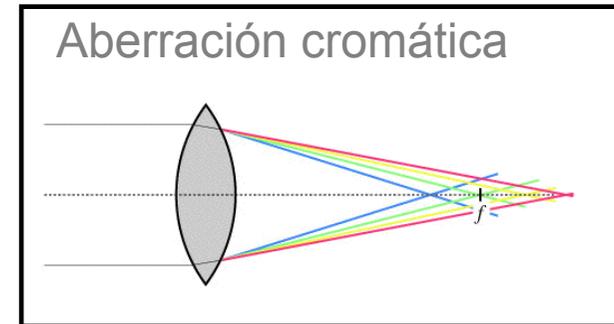
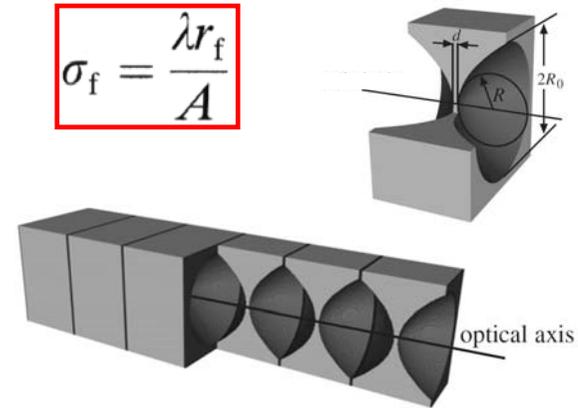
Lentes esféricas



$n = 1 - \delta + i\beta$

10^{-5} Z^3 / E^3

Lentes parabólicas



Ventajas:

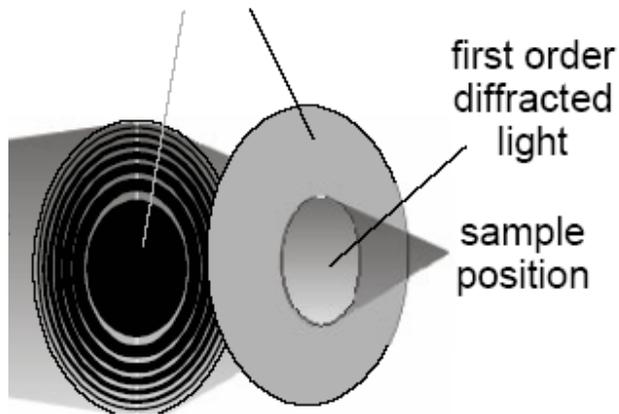
- Resolución (sub)micrométrica
- Amplio rango energético: 5-40 keV
- Fácil de alinear/operar/fabricar (costo)
- Estable bajo intensa carga térmica

Desventajas:

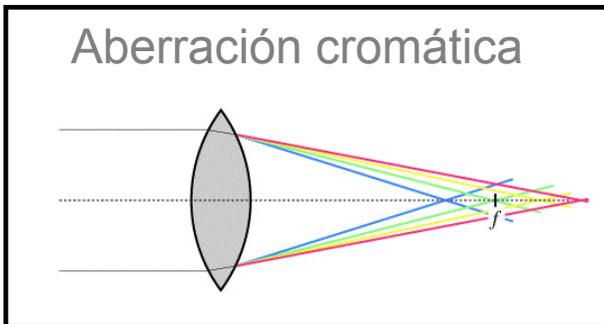
- Pequeña apertura limitada por R (limita el flujo)
- Lentes (Li, Be, C, Al) cromáticas
- Poca focalización (múltiples lentes)
- Baja eficiencia - transmisión



zero order beam stops

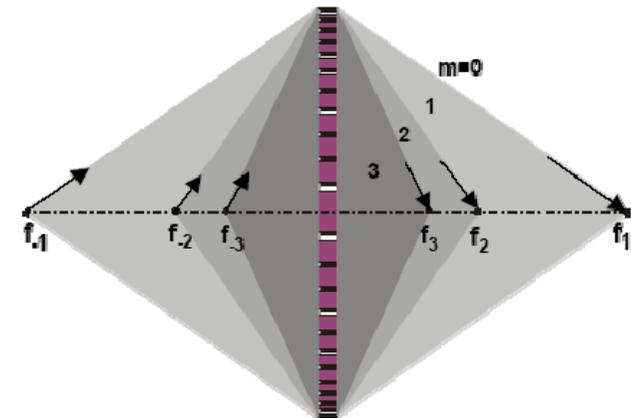
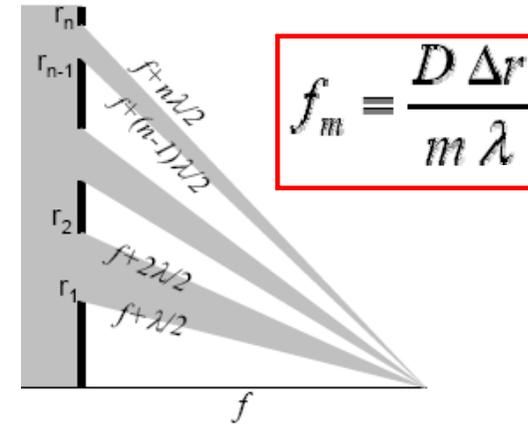
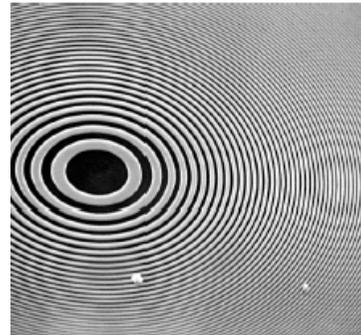


Aberración cromática



Fresnel Zone Plates

$$\delta_m = 1.22 \frac{\Delta r}{m}$$



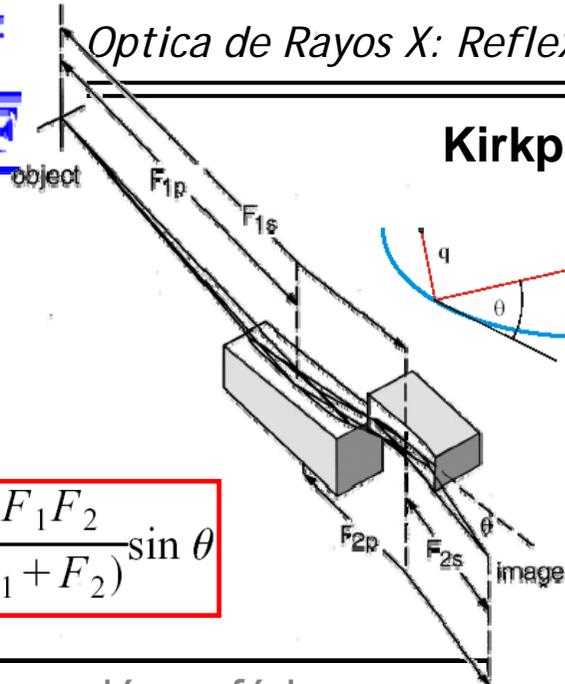
Ventajas:

- Resolución nanométrica (100nm - Δr)
- Rango energético: 250 eV - 15 keV
- Fácil de alinear/operar

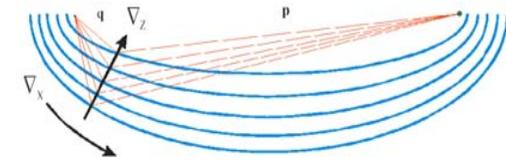
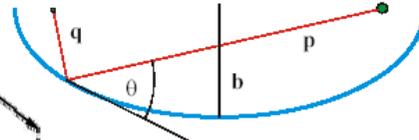
Desventajas:

- Lentes (Au, Ge, Ni on Si, SiN) cromáticas
- Distancia de trabajo corta (mm)
- Apertura limitada por R (poco flujo)
- Baja eficiencia (10% 1er orden difracción)

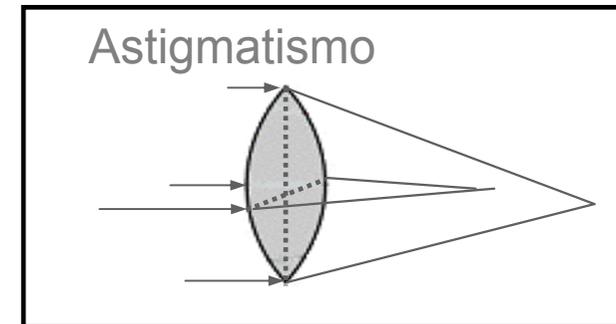
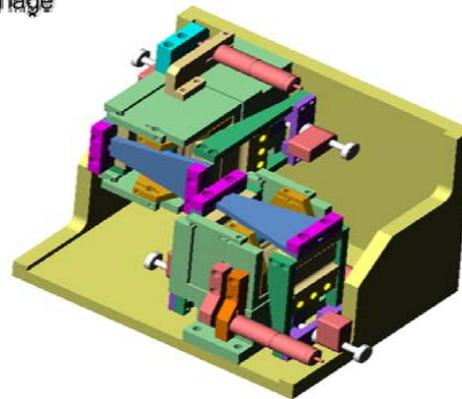
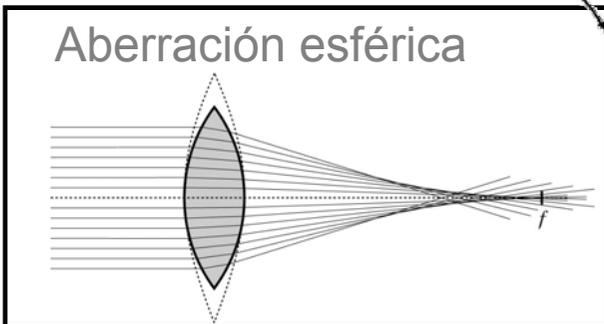
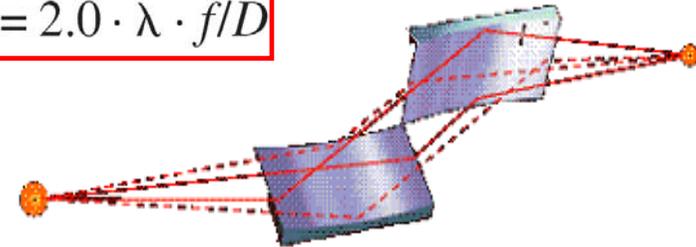
Kirkpatrick-Baez Mirrors



$$R = \frac{2F_1F_2}{(F_1 + F_2)} \sin \theta$$



$$d = 2.0 \cdot \lambda \cdot f/D$$



Ventajas:

- Aberraciones despreciables
- Lente acromática
- Alta eficiencia
- Filtro de altos armónicos

Desventajas:

- Resolución limitada por vibraciones
- Angulo de trabajo crítico muy pequeño
- Sufre inestabilidades térmicas
- Muy sensible a la calidad/pulido de los espejos

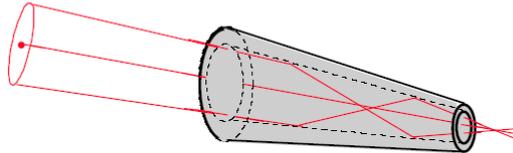
Lentes Bragg-Fresnel



~ 1 x 5 μm^2
A. Snigirev *et al.*,
XRM96 conference, 1996

Difracción

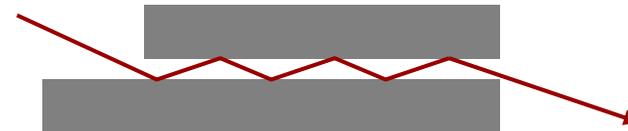
Tapered Capillaries



~ 0.05 x 0.05 μm^2
Bilderback *et al.*,
Science, 263, (1994)

Reflexión

Wave Guides



~ 0.03 x 3 μm^2
Lagomarsino *et al.*

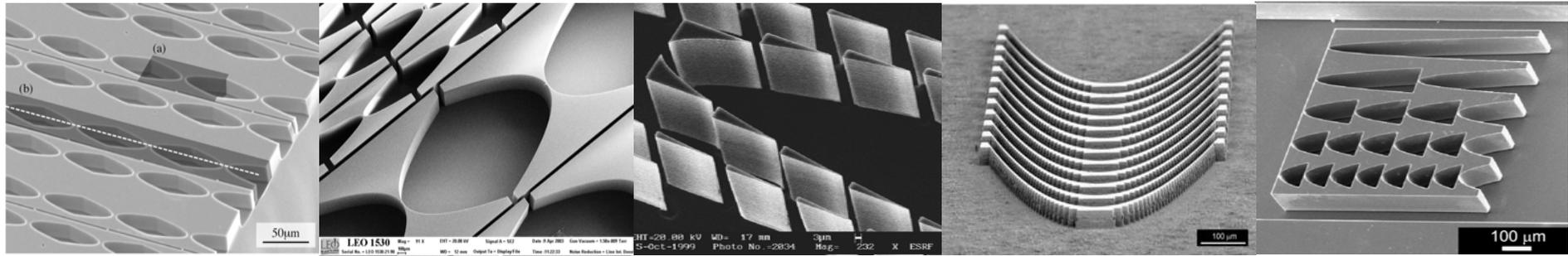
Reflexión

Desventajas:

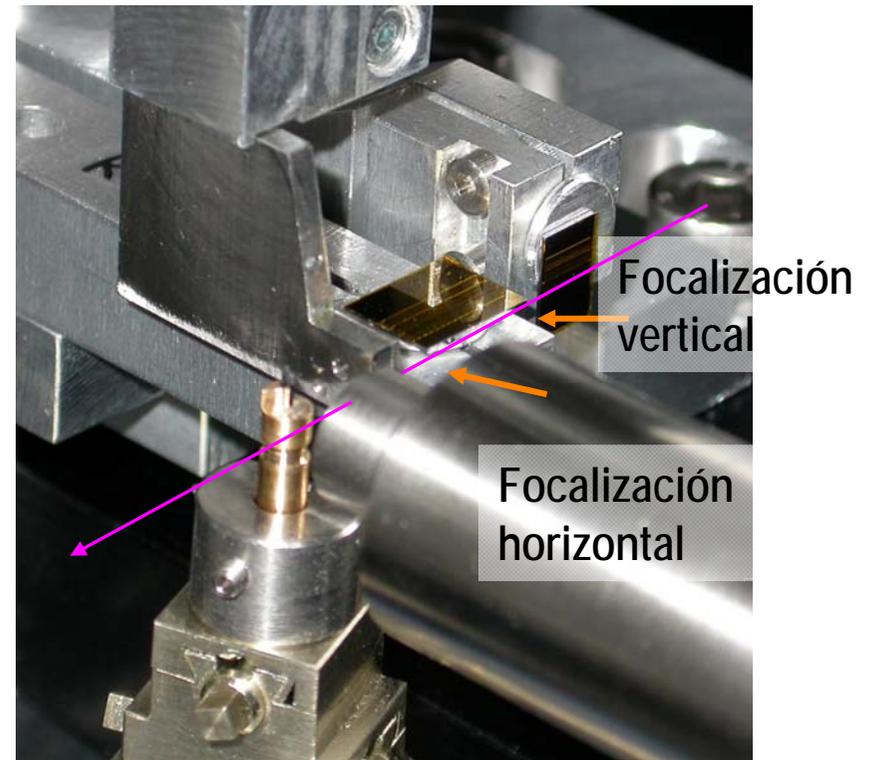
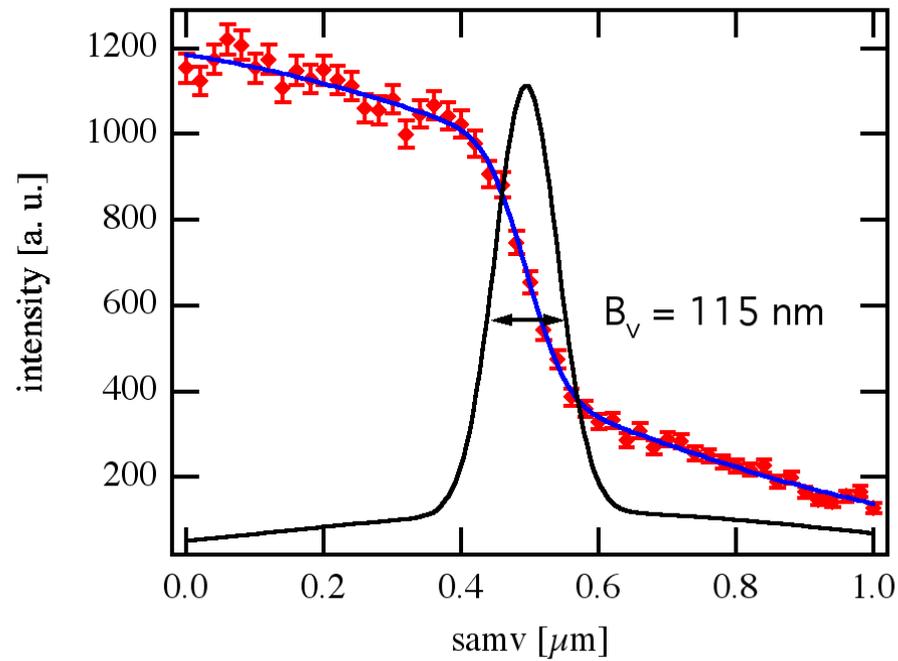
- Difícil de sintonizar E
- Apertura limitada
- Lentes cromáticas

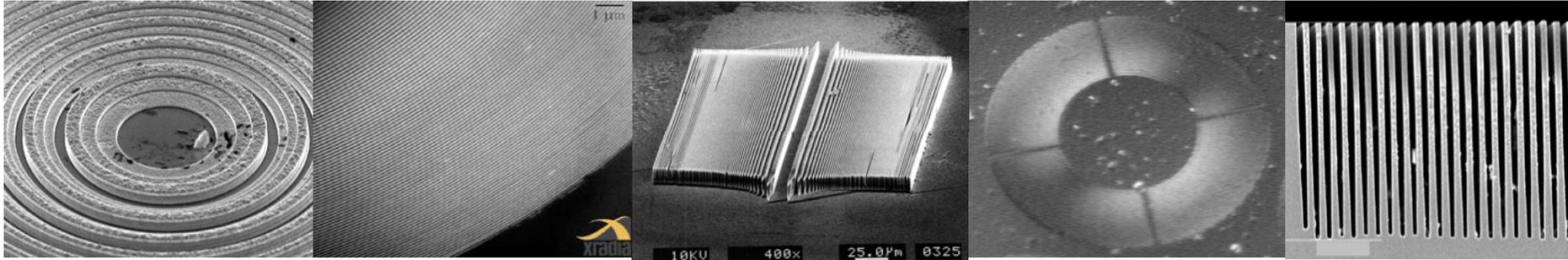
- Vidrio: 2.5 menor θ_c
- No se puede pulir la superficie interna
- Distancia de trabajo muy corta

- Eficiencia muy baja
- Necesita óptica de pre-focalización
- Sufre aún imperfecciones (interfaz, homogeneidad)

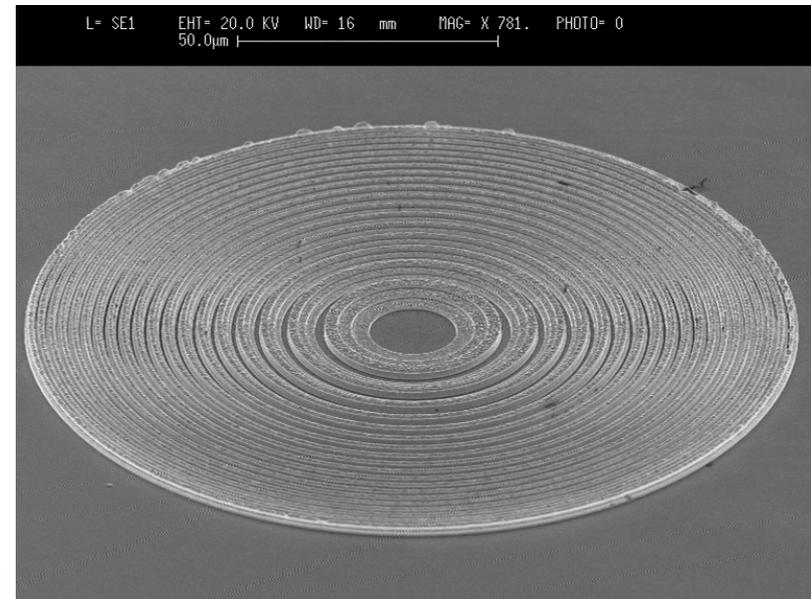
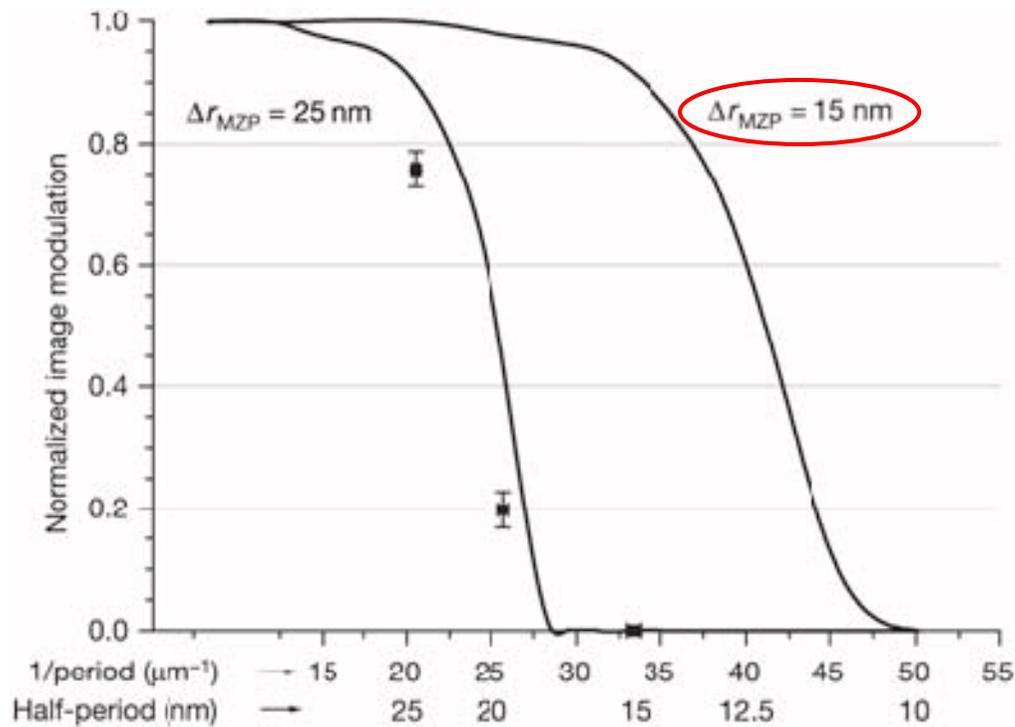


Lentes Cruzadas de Si

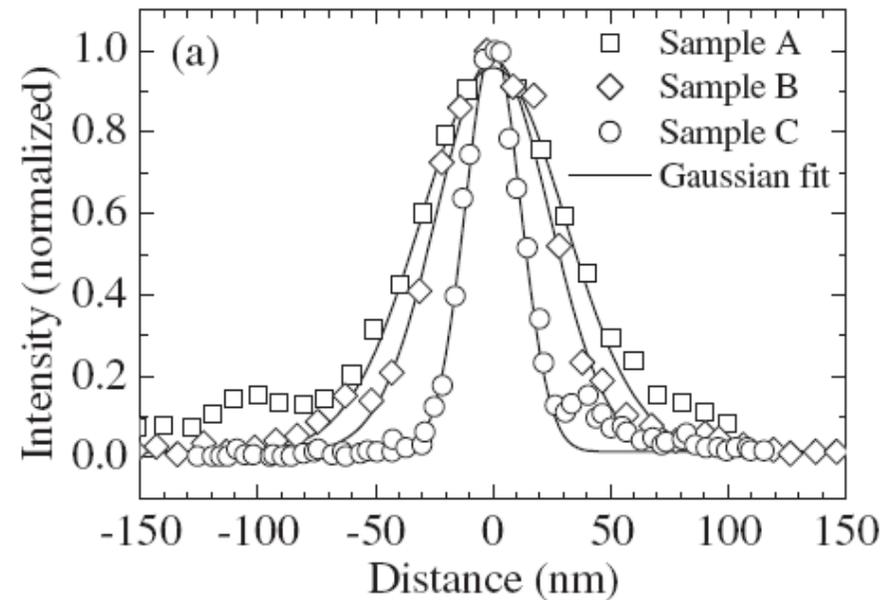
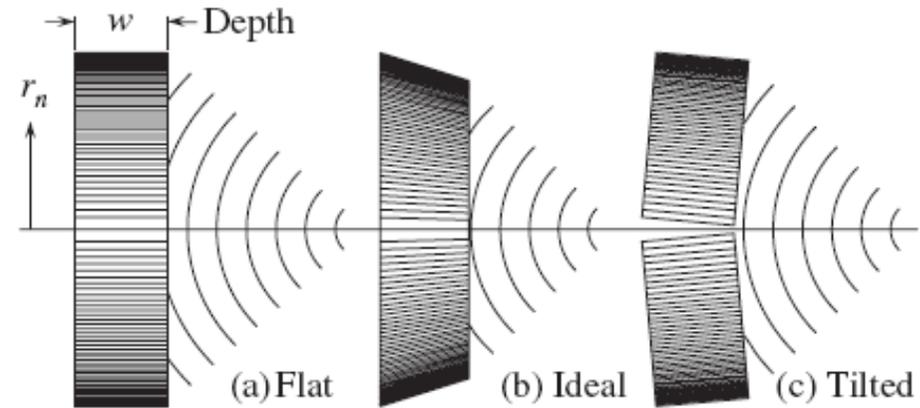
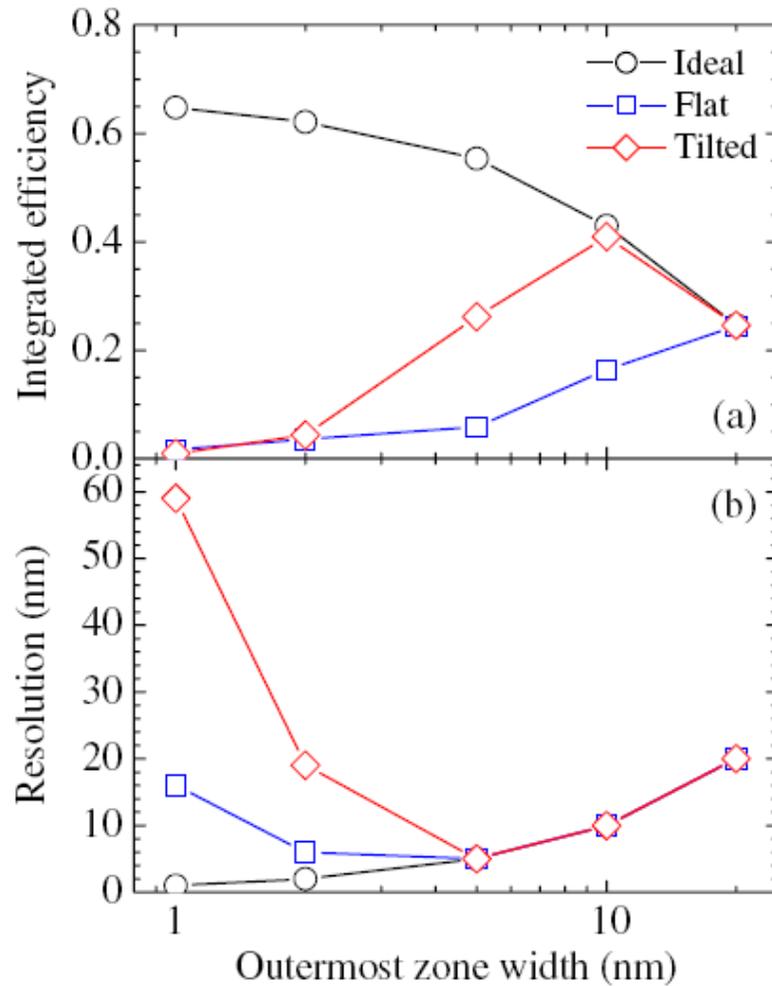


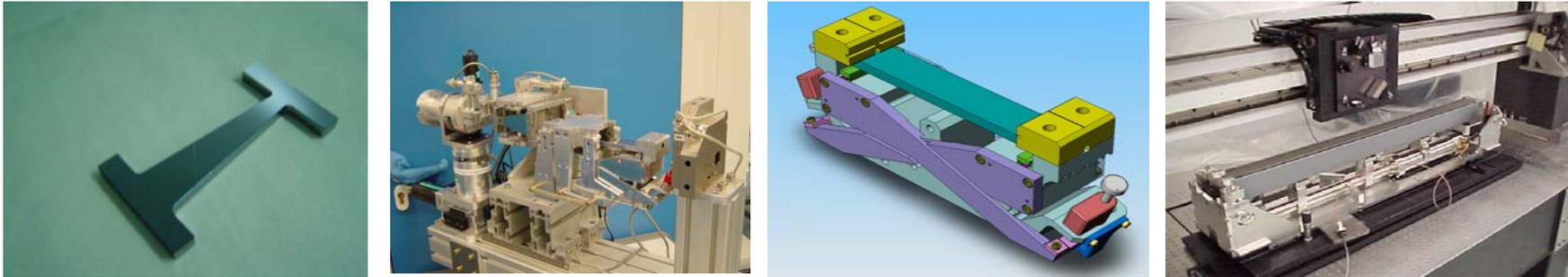


Fresnel Zone Plates

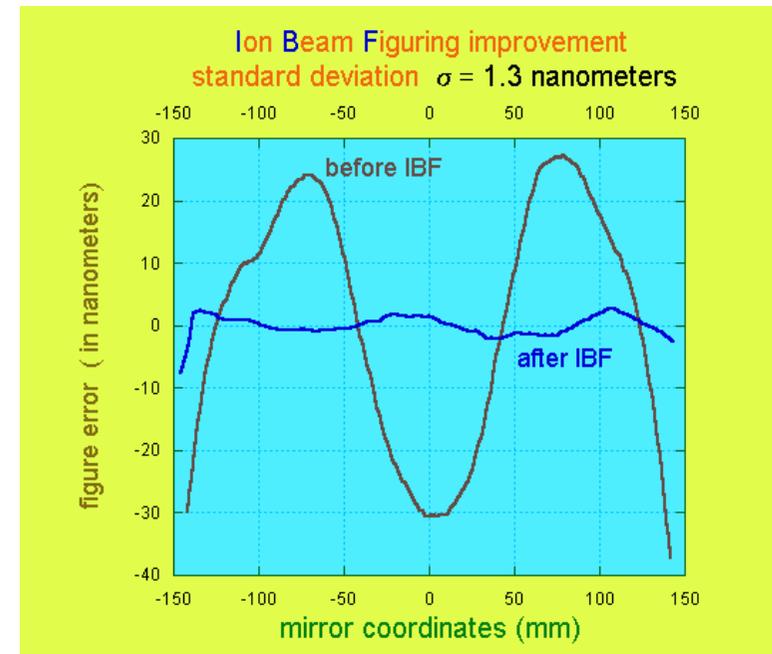
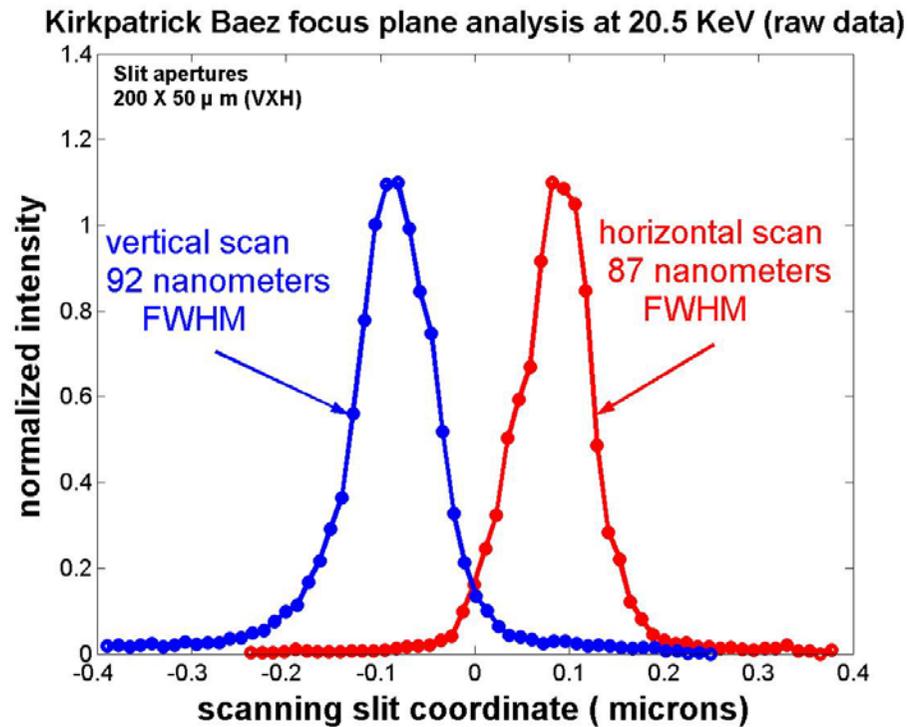


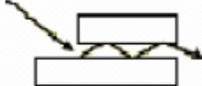
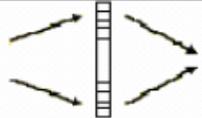
Multilayer Laue Lens



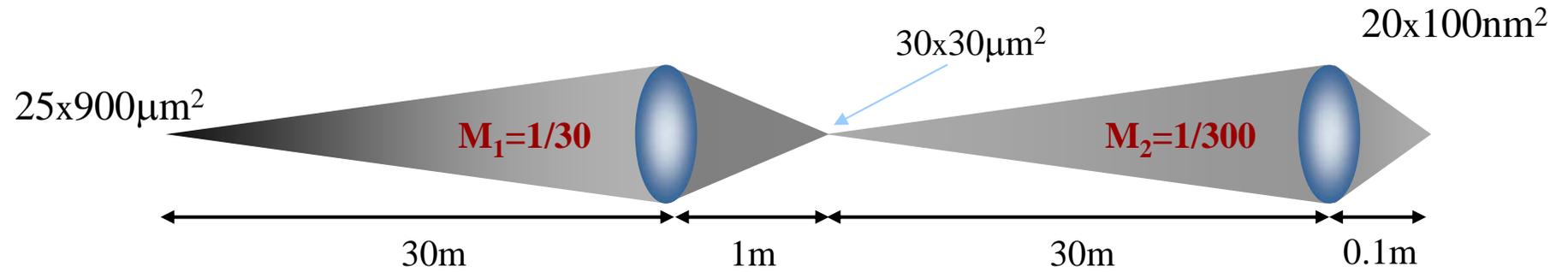


Espejos - configuración Kirkpatrick-Baez - ID22NI

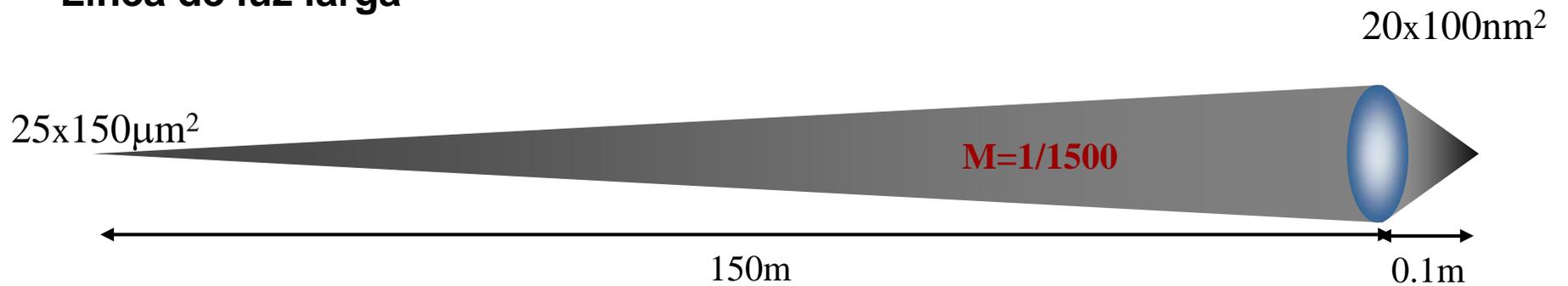


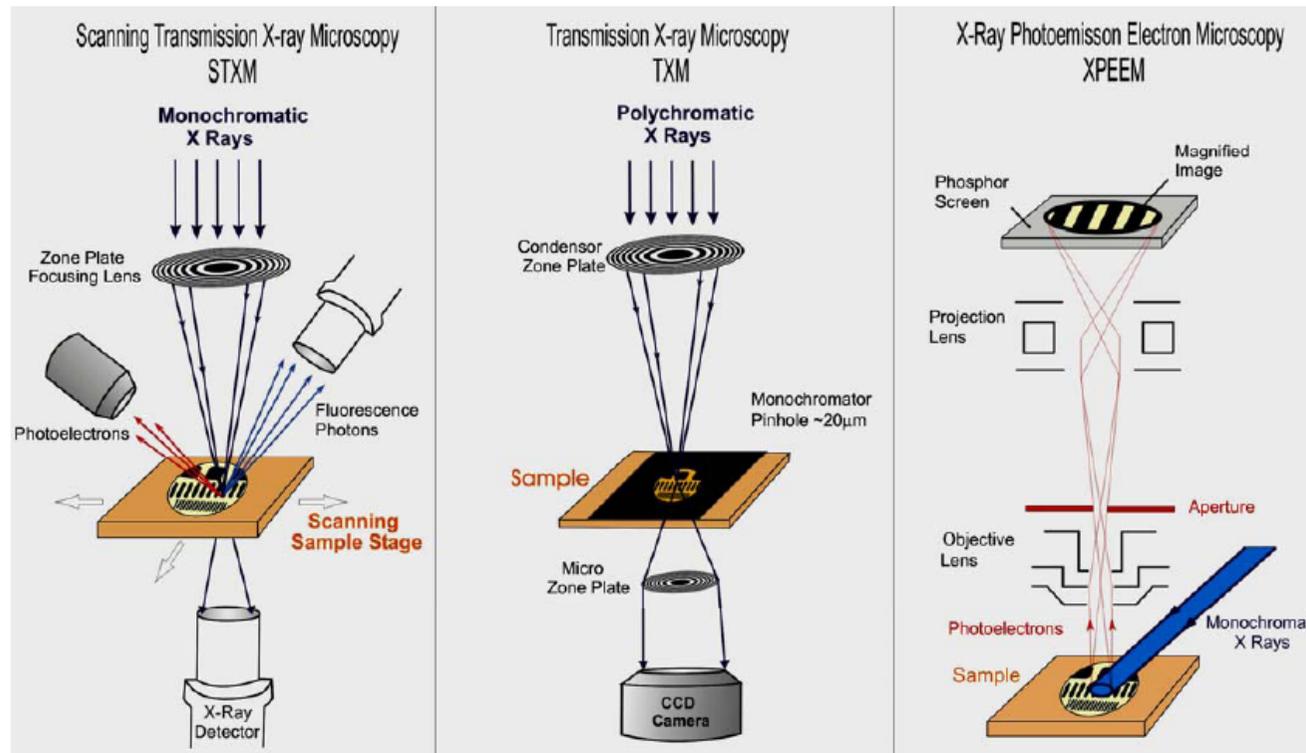
	REFLEXION				DIFRACCION	REFRACCION
	Sistema Kirkpatrick-Baez		Capilares	Guías de Onda	Lentes Zonales de Fresnel	Lentes Refractivas
	Espejos K. & Baez, 1948	Multicapas Barbee, 1986	Kreger, 1948	Feng, 1993	Baez, 1952	Snigirev, 1986
						
E	<30 keV	<80keV	<20keV	<20keV	<30keV (80)	<1MeV
$\Delta E/E$	Ancho de Banda Amplio	10^{-2}	Ancho de Banda Amplio	10^{-3}	10^{-3}	10^{-3}
Resolución	25 nm (15keV)	41 x 45nm² (24 keV)	50 nm	40 x 25 nm²	30nm (20 keV) 15nm (<1keV)	50nm (20keV) 150nm (50keV)
	Mimura (2006)	Hignette (2005)	Bilderback (1994)	Salditt (2004)	Kang (2006)	Schroer (2004) Snigirev (2006)
Eficiencia	+++	+++	- - -	- - -	++	+
Dimensión	+++	+++	+++	+++	+++	+++
Acromática	SI	NO	SI	NO	NO	NO
Coherencia	+	+	+/-	+++	++	+/-
Foc en-línea	NO	NO	SI	SI	SI	SI
Dist.F. Larga	SI	SI	NO	NO	SI	SI
Complejidad	+/-	+/-	++	+/-	++	++

Uso de fuente auxiliar



Línea de luz larga

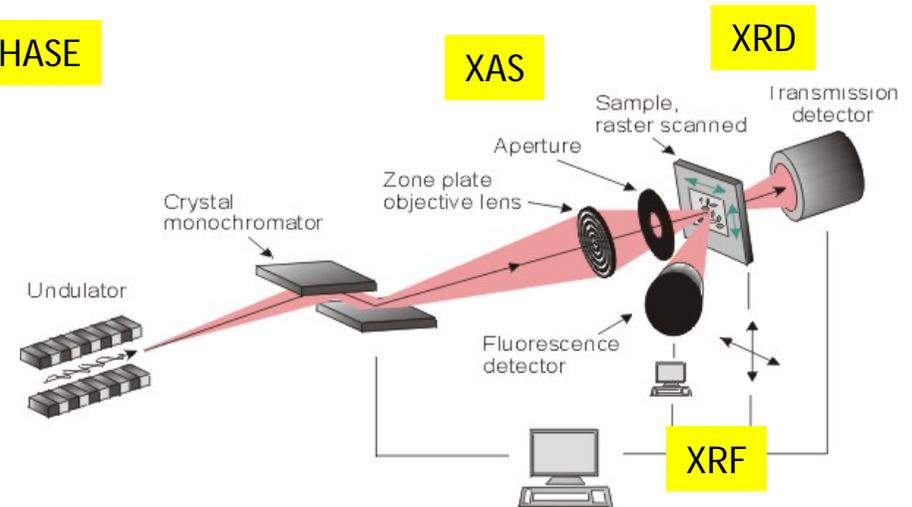
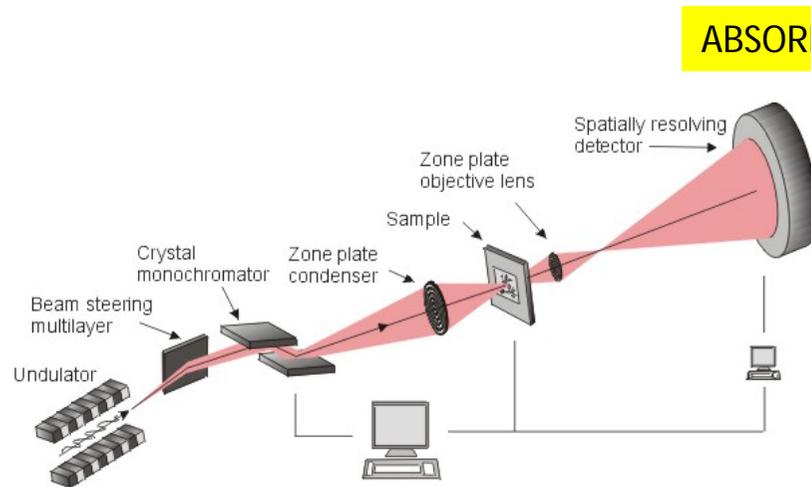




TYPE OF MICROSCOPE	ENERGY (keV)	MODE OF CONTRAST	OPTICS	RESOLUTION (nm)
Full field Microscopy				
Micro-radiography	> 0.5	Absorption	-	100 - 1000
Contact In-line imaging	> 1	Absorption/phase, XAS	-	500 - 1000
Magnified Projection (no optics)	> 1	Absorption/phase	-	100 - 500
Soft X-ray Microscopy	< 4	Amplitude/phase, XAS	FZP	20 - 50
Hard X-ray Microscopy	> 4	Amplitude/phase, XAS	FZP, CRL	100 - 500
Scanning Microscopy				
Soft X-ray Microscopy	< 4	Absorption, XRF, XAS, XPEEM	FZP	20 - 50
Hard X-ray Microprobe	> 4	Absorption, XRF, XAS, XRD	FZP, CRL, KB	100 - 1000

Full-field X-ray microscope

Scanning X-ray microscope



Ventajas:

- Rápido y eficaz para tomografía
- Instrumentación relativ. simple
- Mayor resolución espacial

Desventajas:

- Dosis ineficiente
- Sólo se detecta haz transmitido

Ventajas:

- Dosis eficiente
- Múltiple detección en paralelo
- Aplicable a material volumétrico

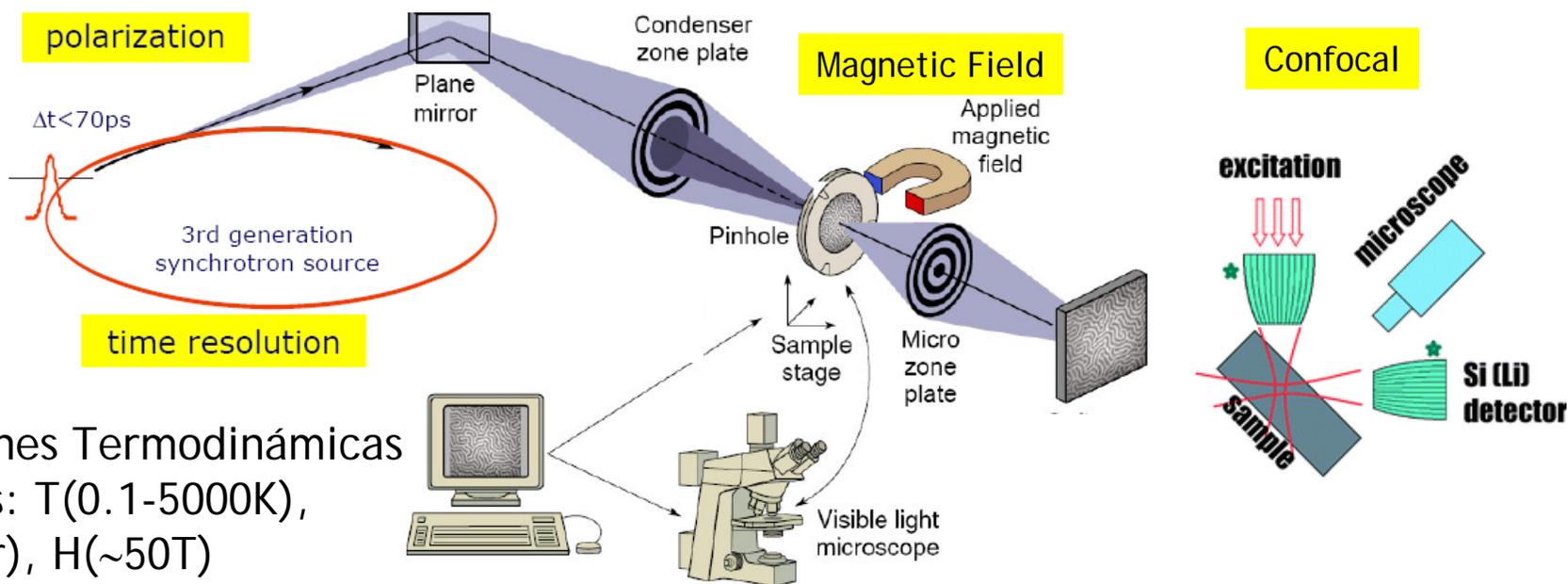
Desventajas:

- Técnica lenta
- Instrumentación compleja



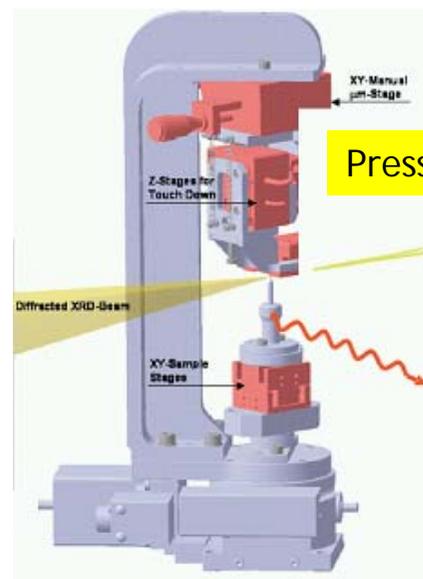
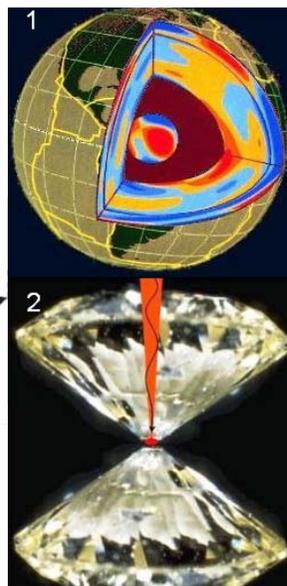
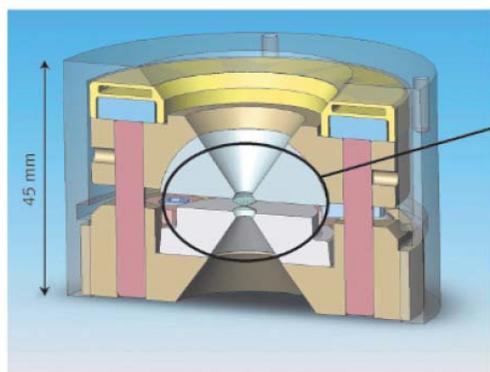
Microscopios de Rayos X: In-situ

Instrumentación

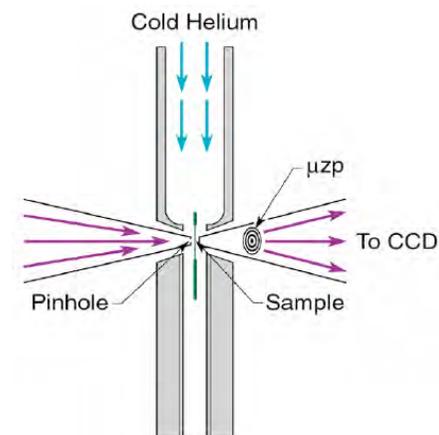


Condiciones Termodinámicas
Extremas: T(0.1-5000K),
P(~3Mbar), H(~50T)

High pressure and temperature



Low temperature



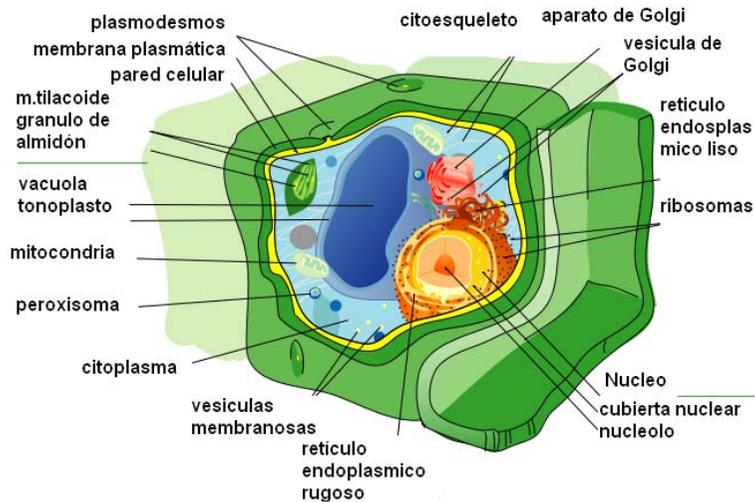


Indice

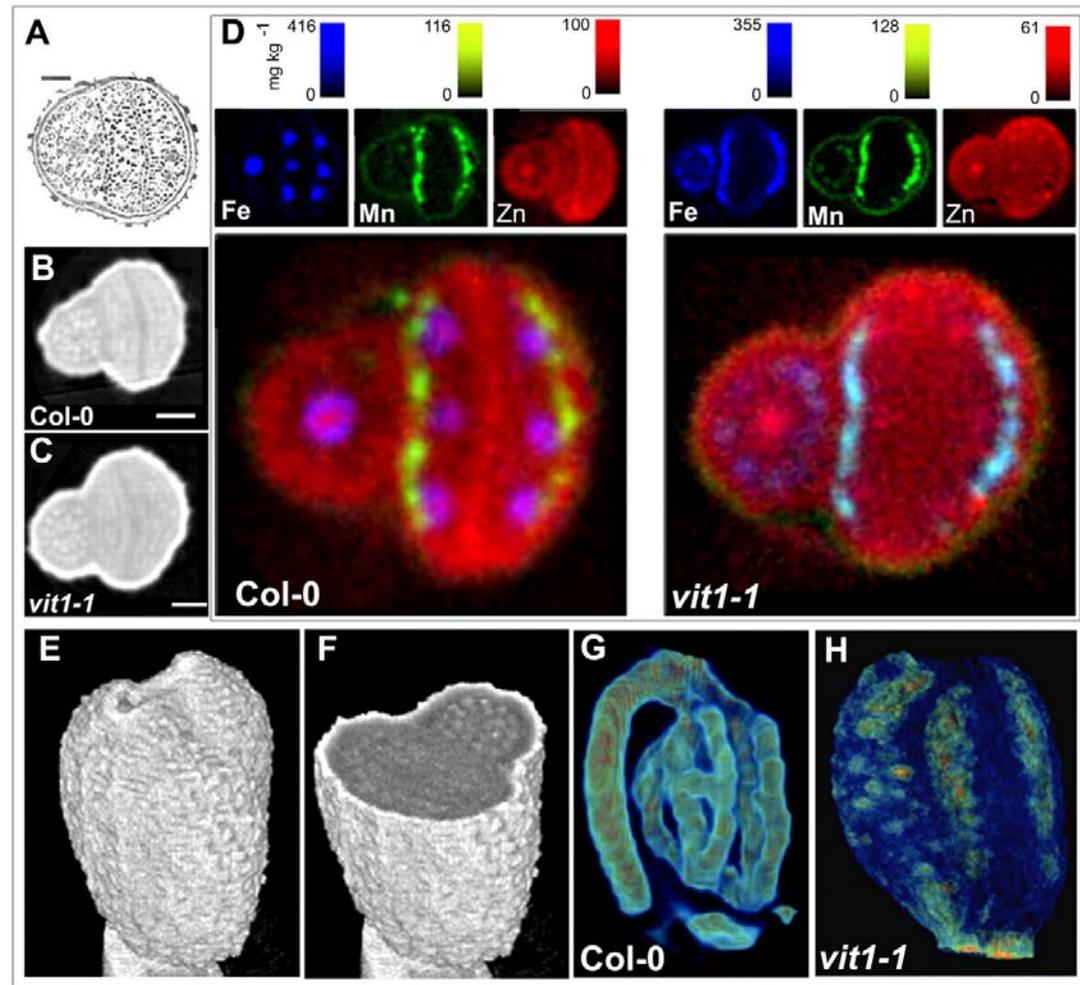
- Principios: microscopía de rayos X
 - Introducción
 - Radiación Sincrotrón
 - Propiedades de los Rayos X
- Instrumentación: técnicas microscópicas
 - Óptica de Rayos X
 - Estrategias de Nanofocalización
 - Microscopios de Rayos X: modos de operación
- **Aplicaciones**

Hierro: Localización en la semilla de Arabidopsis: transportador vacuolar

Arabidopsis



Science 314. pp. 1295 – 1298 (2006) by Kim et al

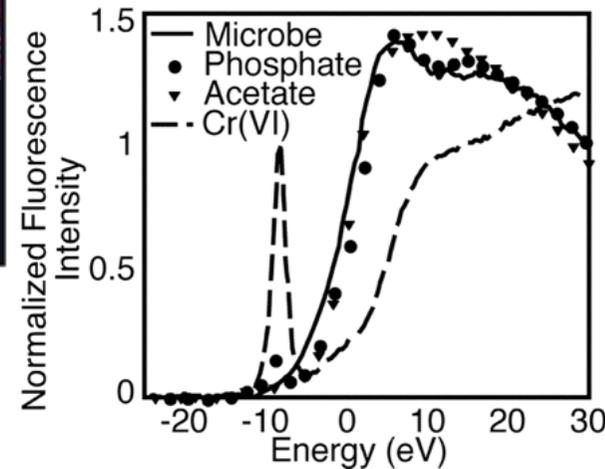
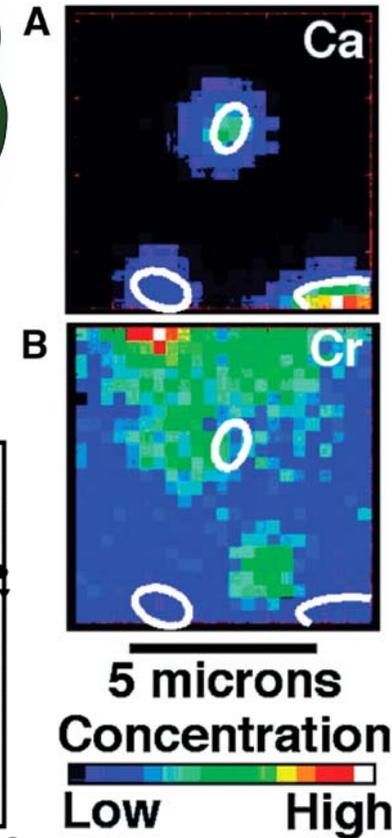
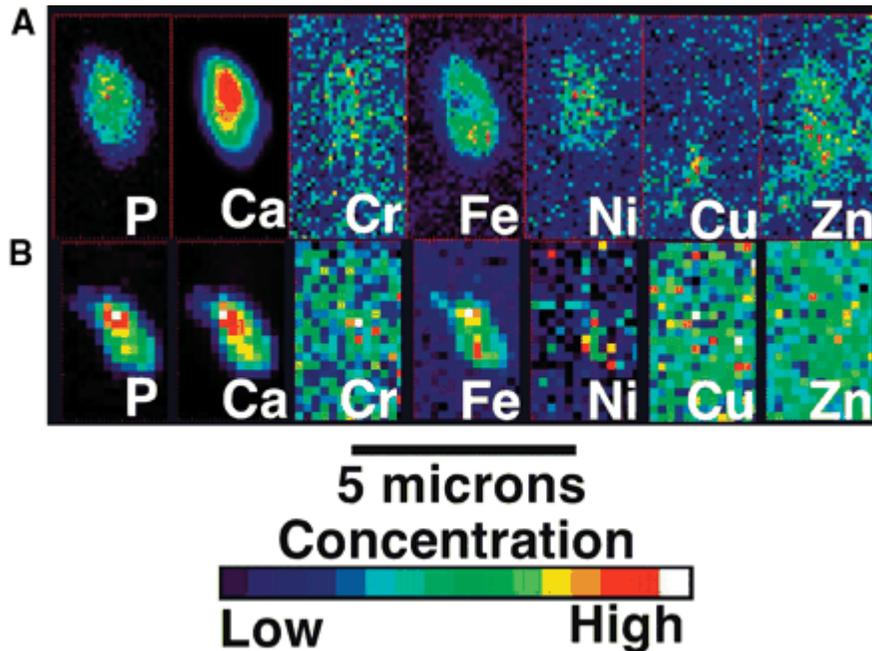
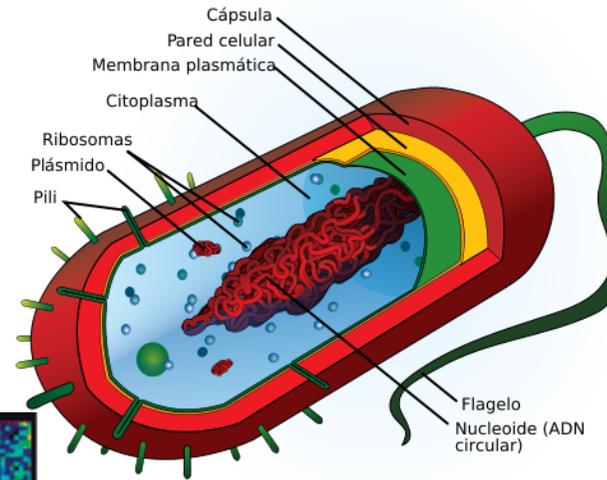


Análisis elemental y redox de células bacterianas

Science 306, pp. 686-687 (2004) by Kemner *et al*

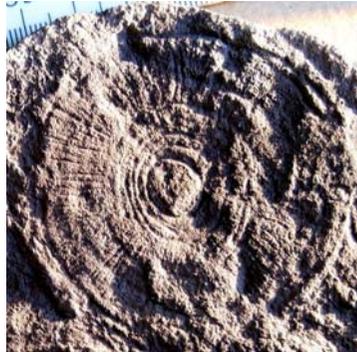


Protista

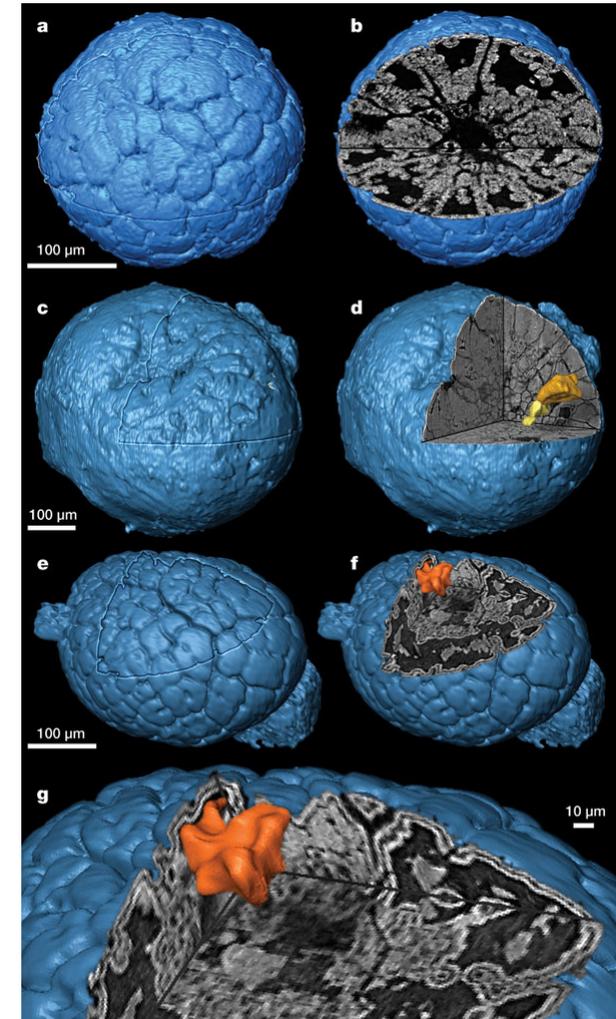
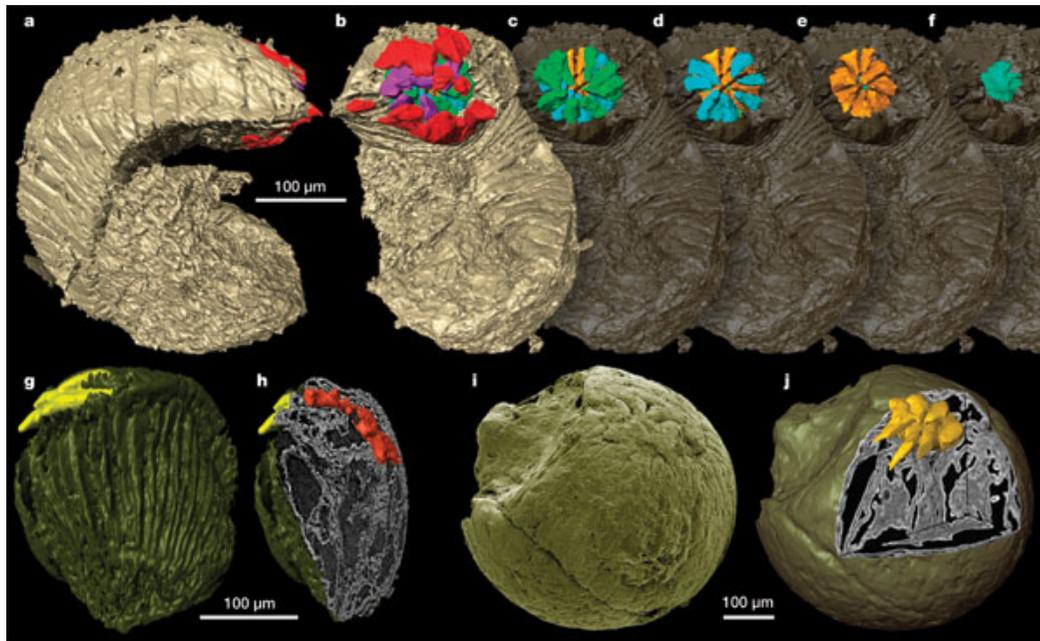


Microscopía tomográfica de fósiles embrionarios

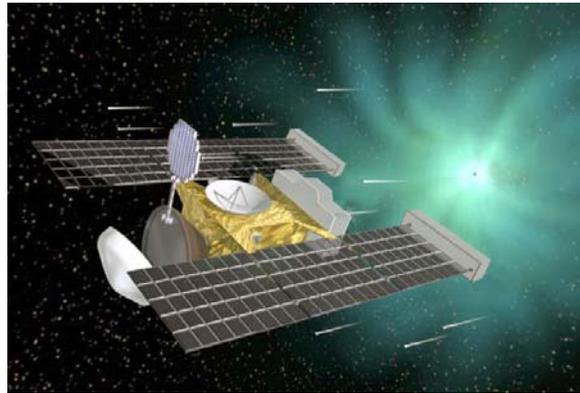
Nature 442. pp. 680-683 (2006) by Donoghue *et al*



Fósiles

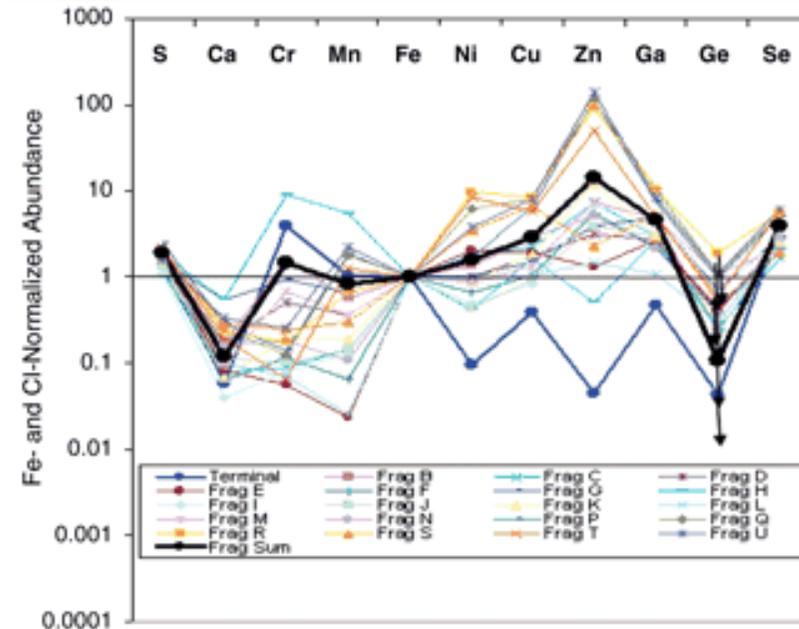
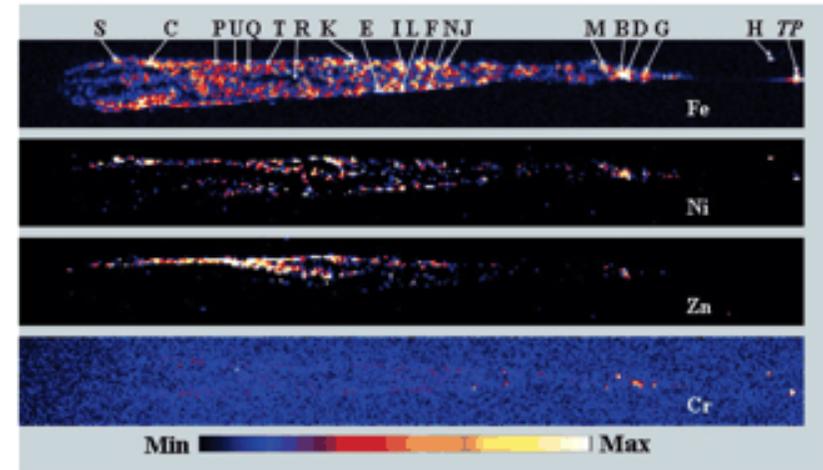
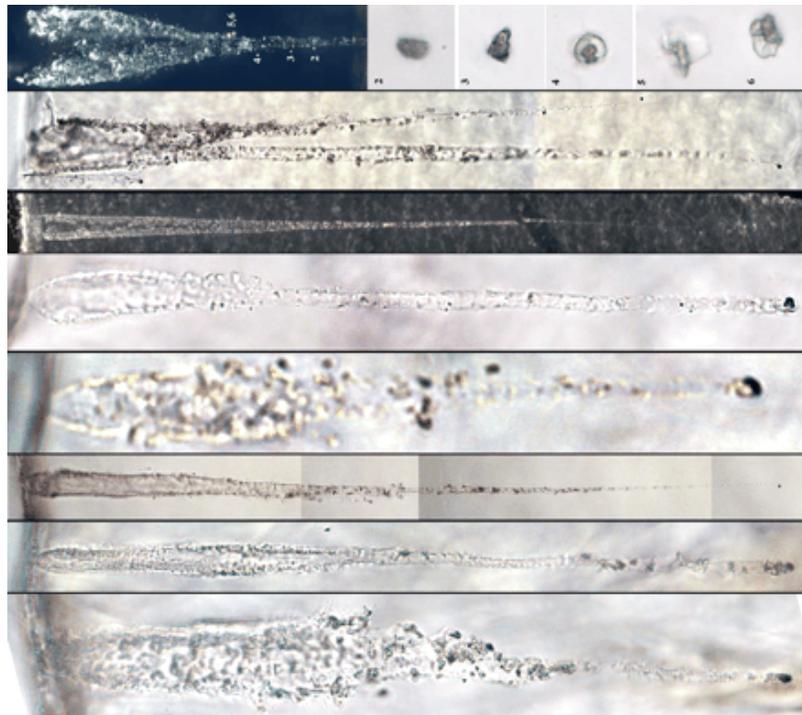


Composicion elemental - cometa 81P/Wild 2 - STARDUST



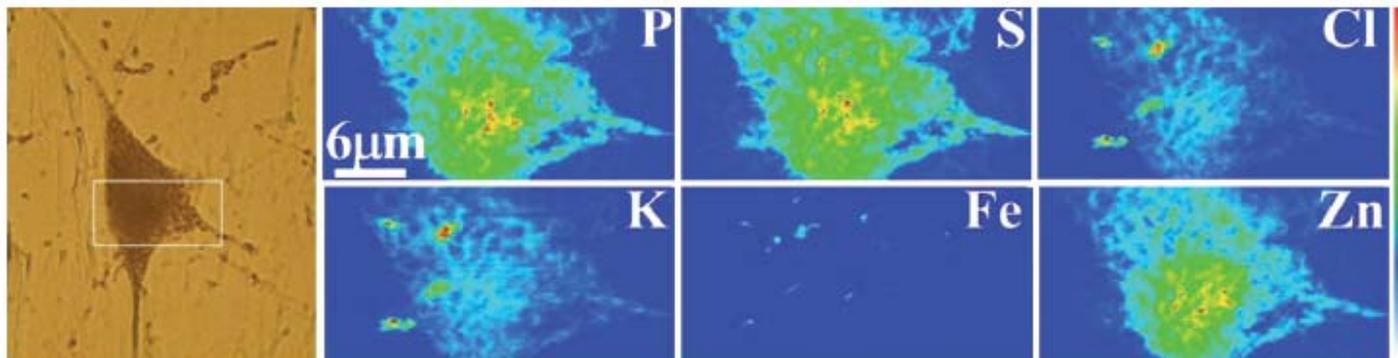
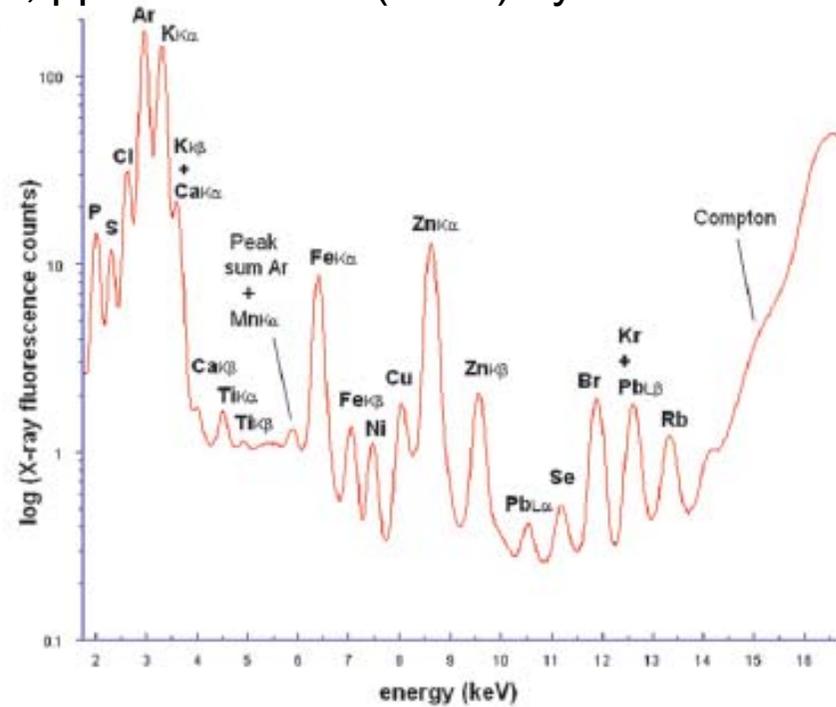
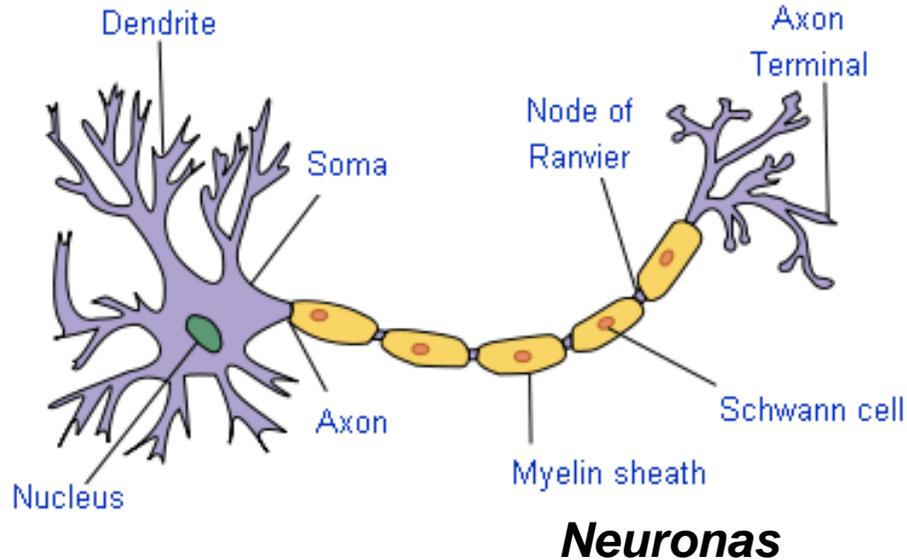
Particulas Extraterrestres

Science 314, pp. 1731-1735 (2005) by Flynn et al



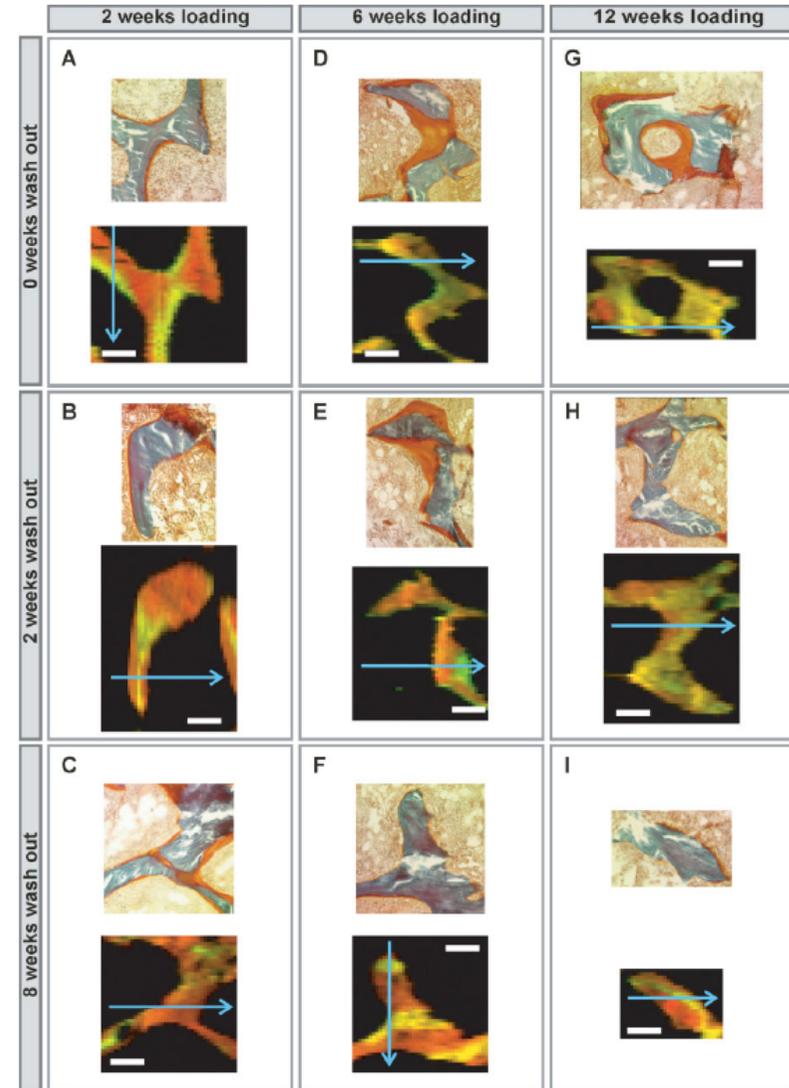
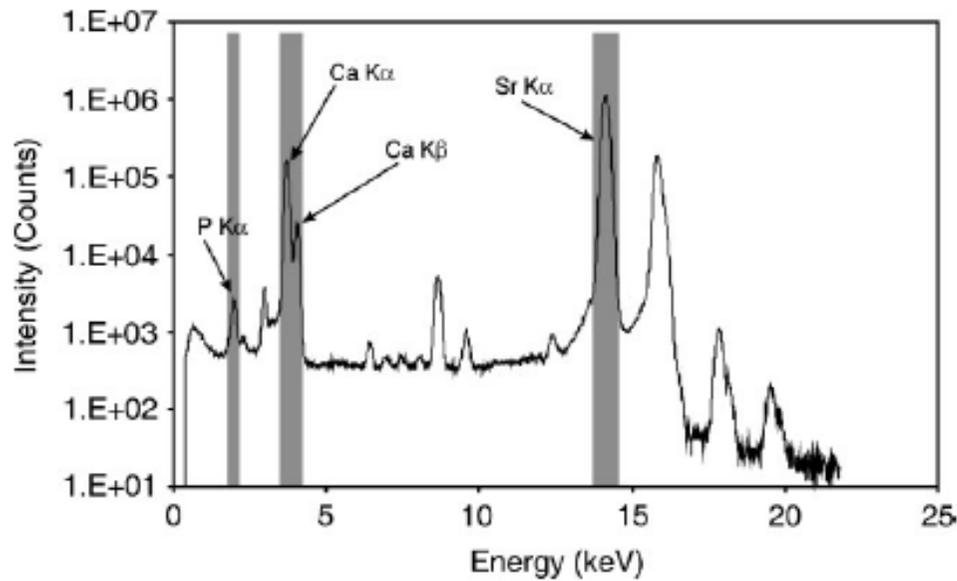
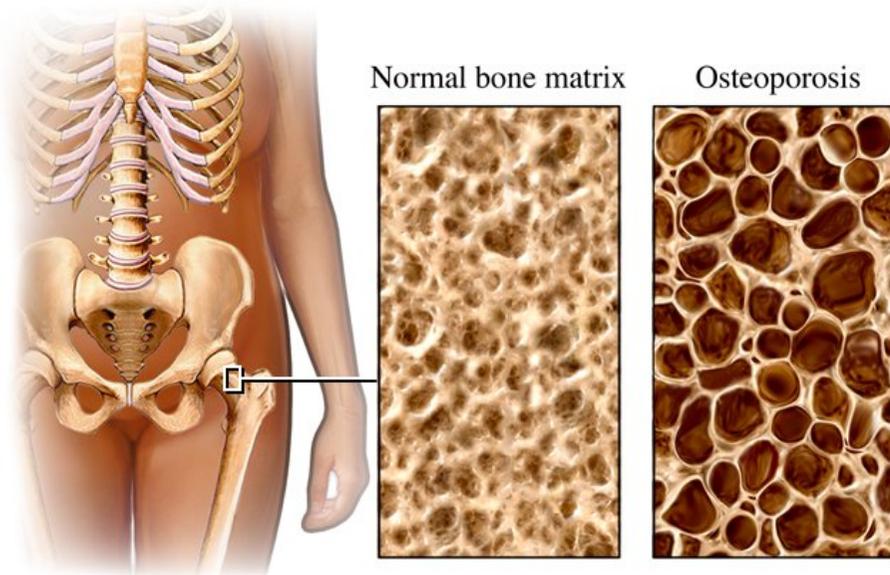
Hierro - Enfermedad de Parkinson

JAAS 23, pp. 1045-1168 (2008) by Carmona *et al*

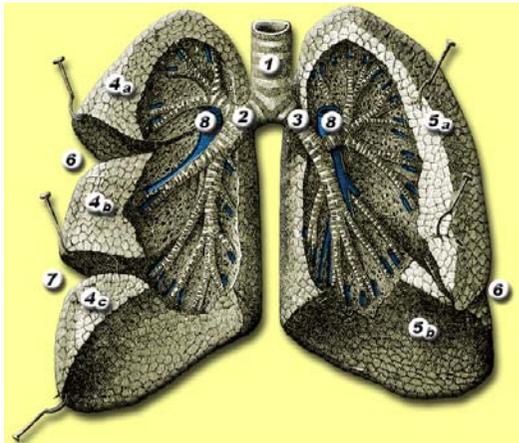


Estroncio - Agente terapeutico en la osteoporosis

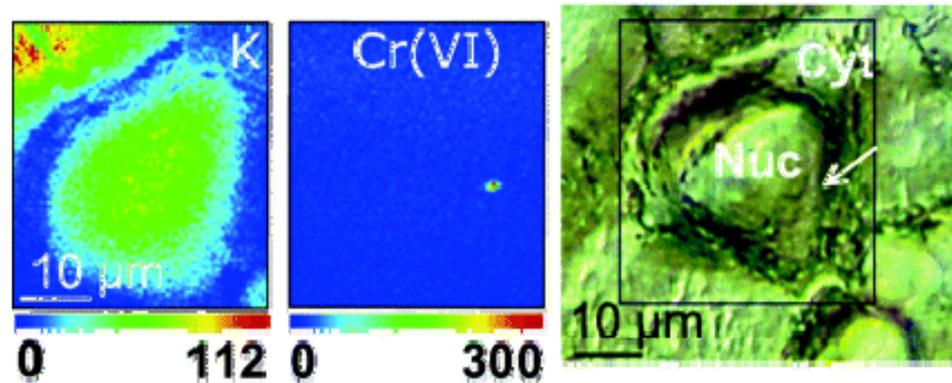
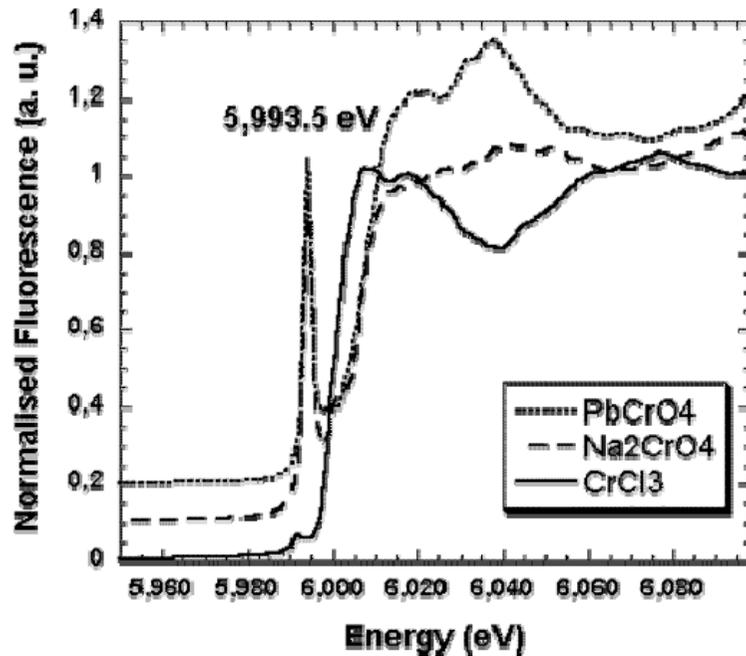
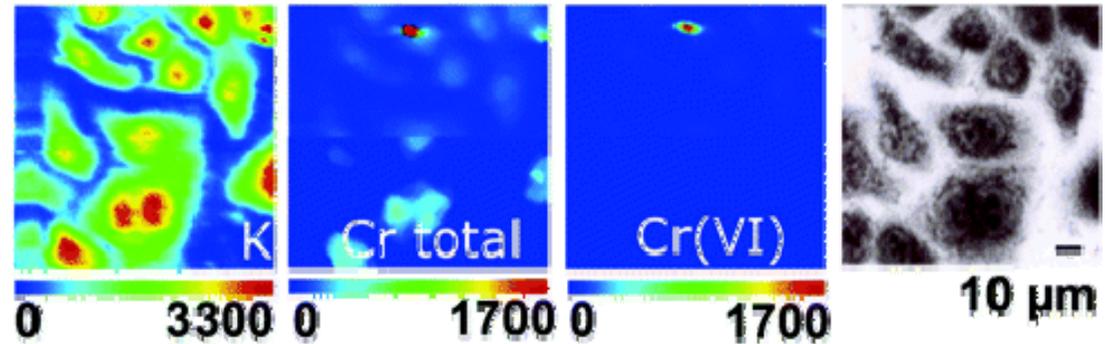
XRS 36, pp. 42-49 (2007) by Oste *et al*



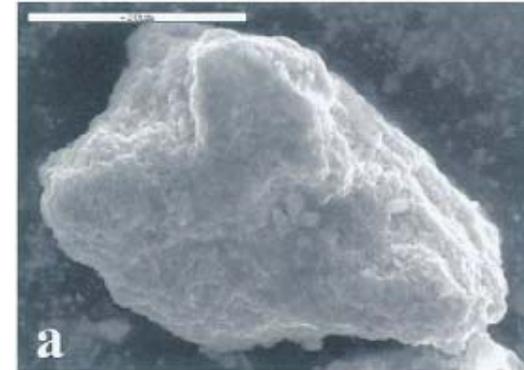
Cr (VI) - Agente carcinogeno para las vias respiratorias



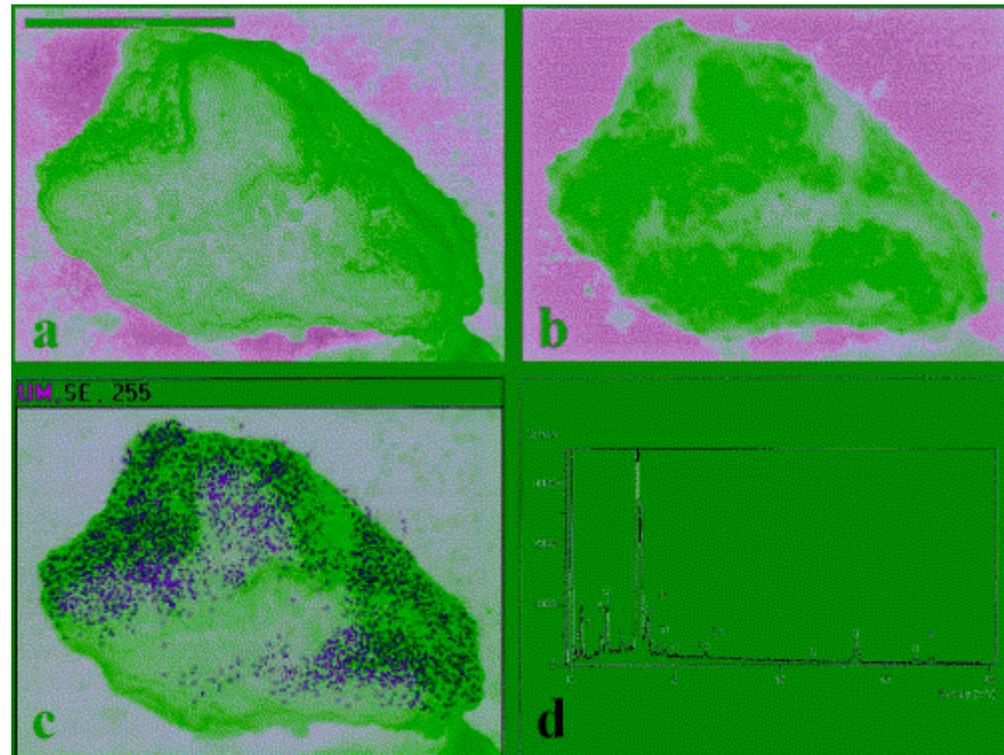
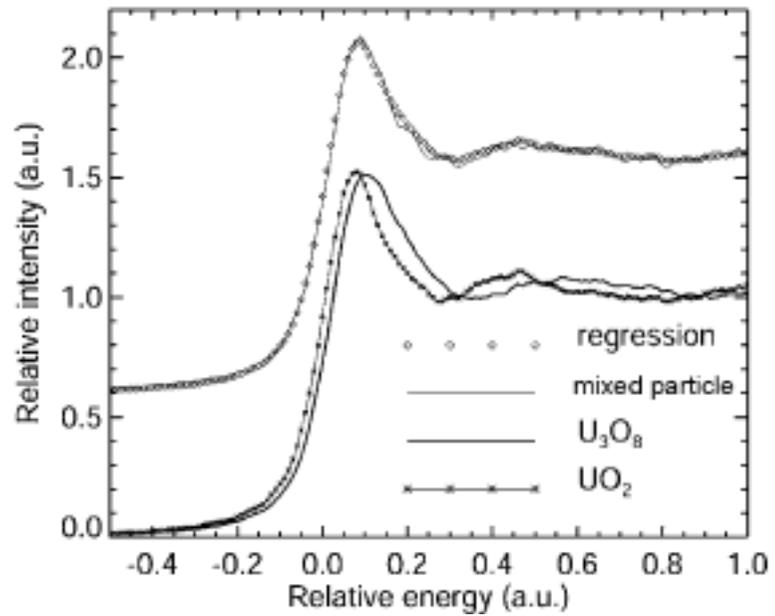
CRT 18, pp. 1512-1519 (2005) by Ortega *et al*



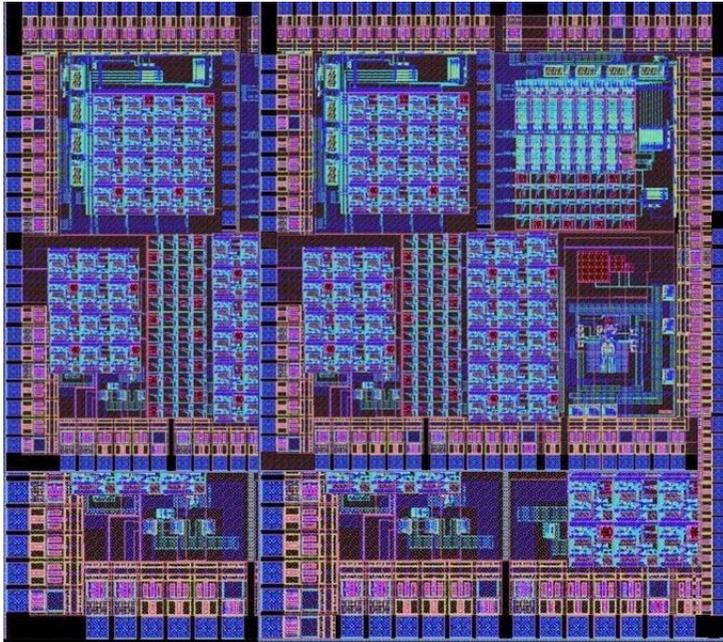
Uranio Empobrecido - Chernobyl



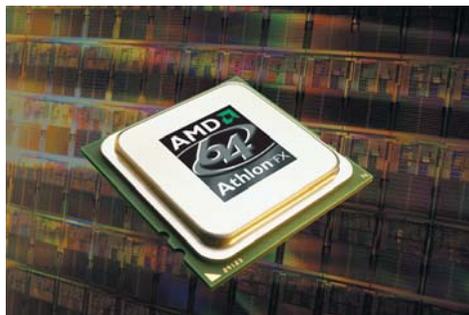
JER 64, pp. 167-173
(2003) by Salbu *et al*



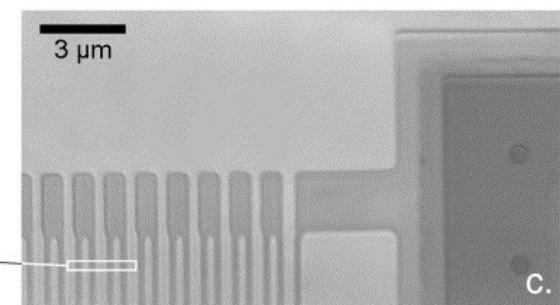
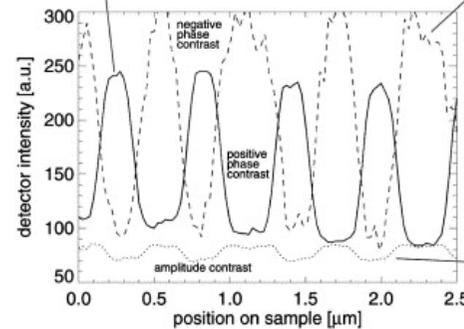
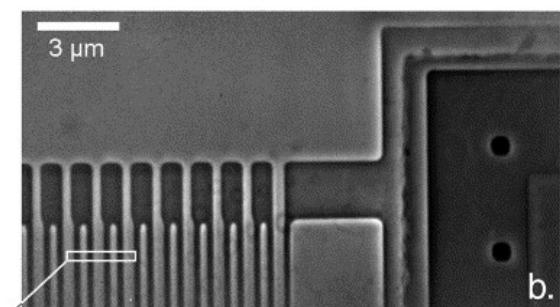
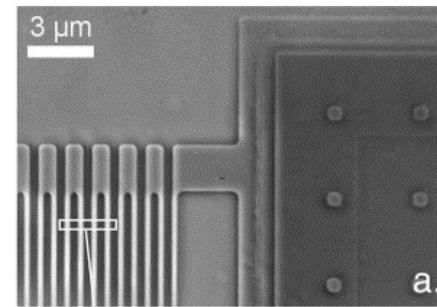
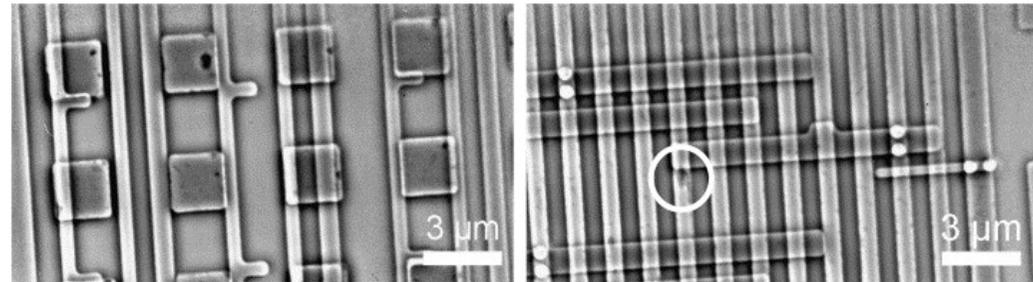
ULSI - Ultra Large Scale Integration



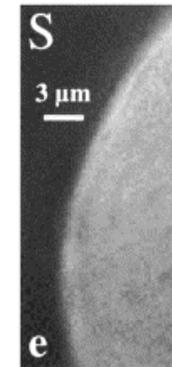
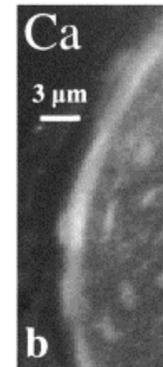
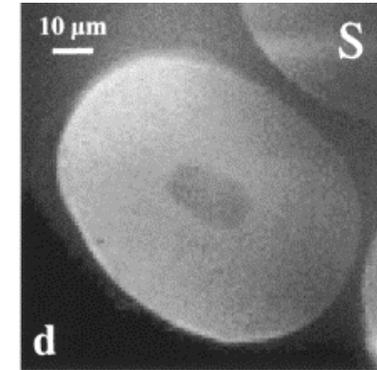
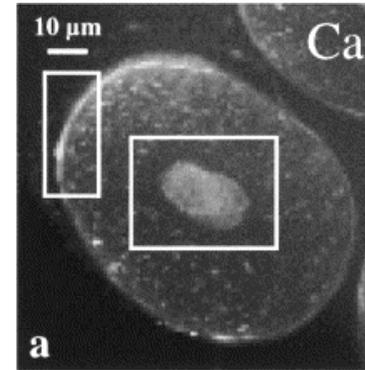
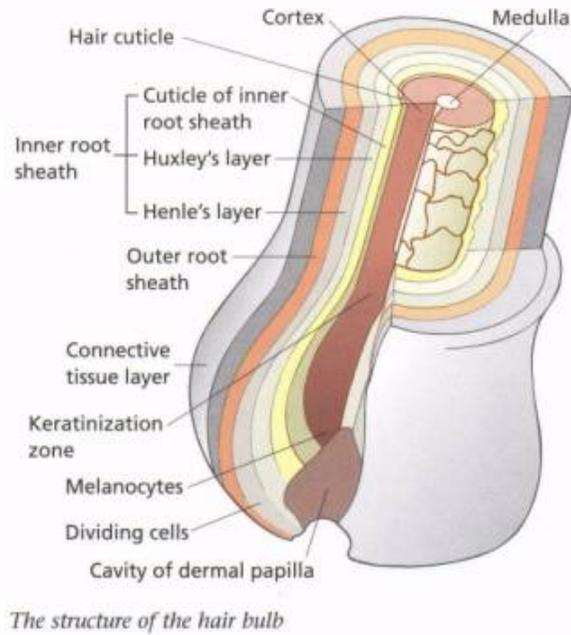
Numero de componentes:
Igual o superior a 100 000



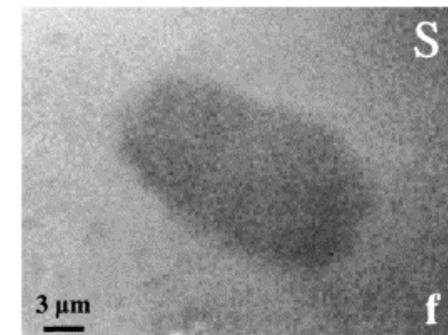
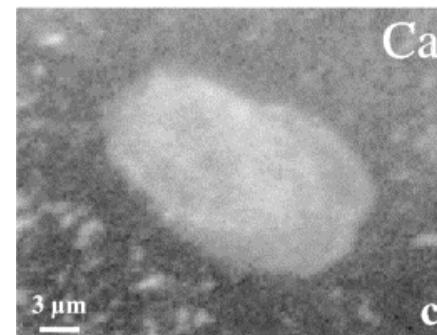
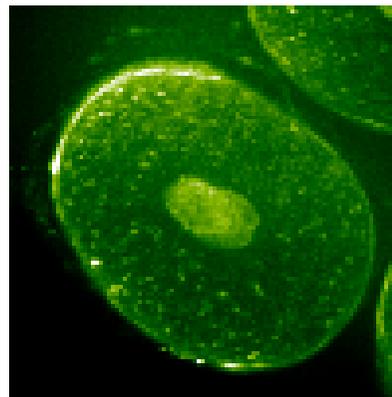
ME 83, pp. 1043-1046
(2006) by Neuhausler *et al*



Calcio – pelo del cuero cabelludo

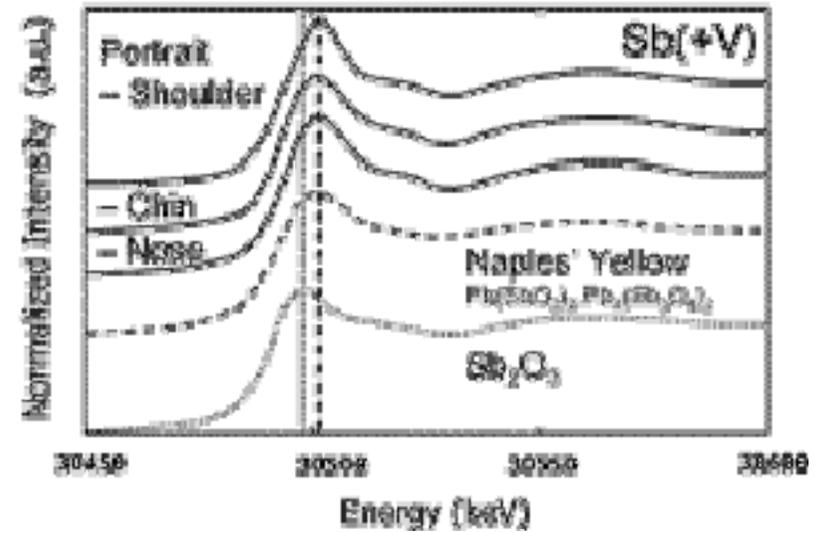
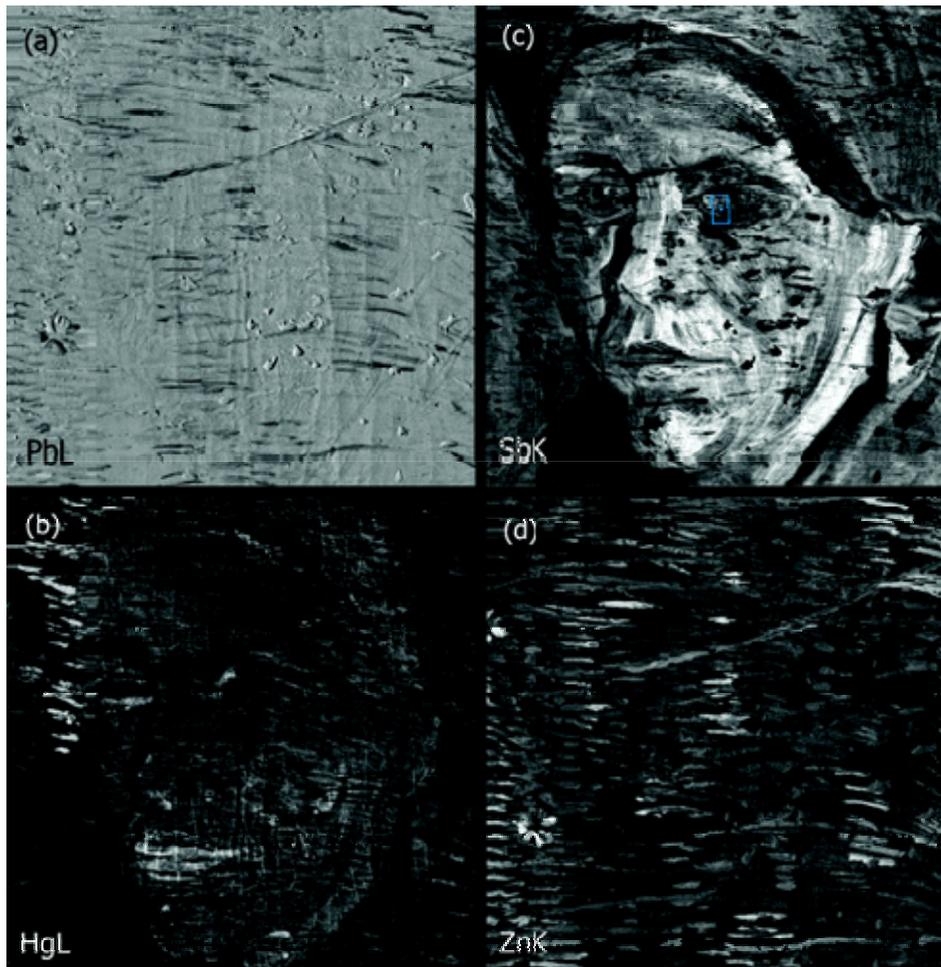


BBA 1619, pp. 53-58 (2003) by Merigoux et al



Sb, Hg – Vincent van Gogh

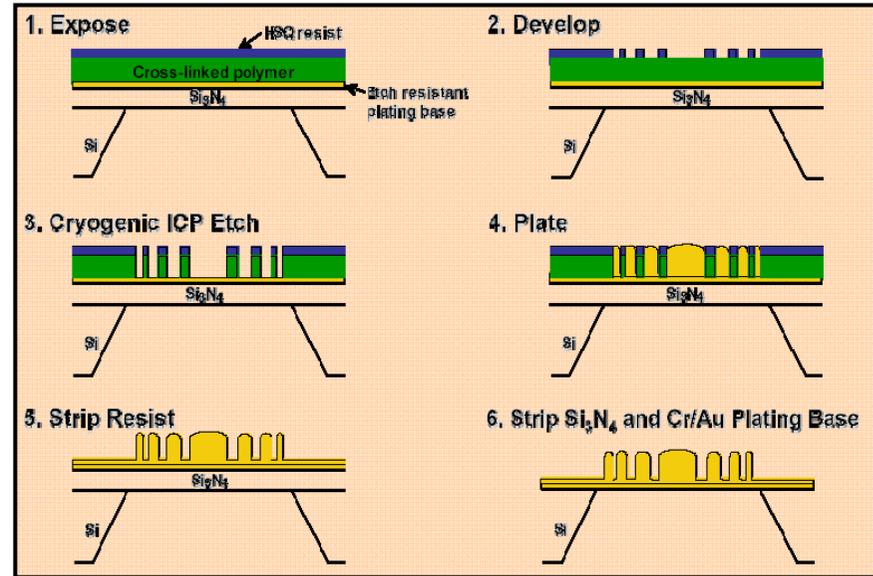
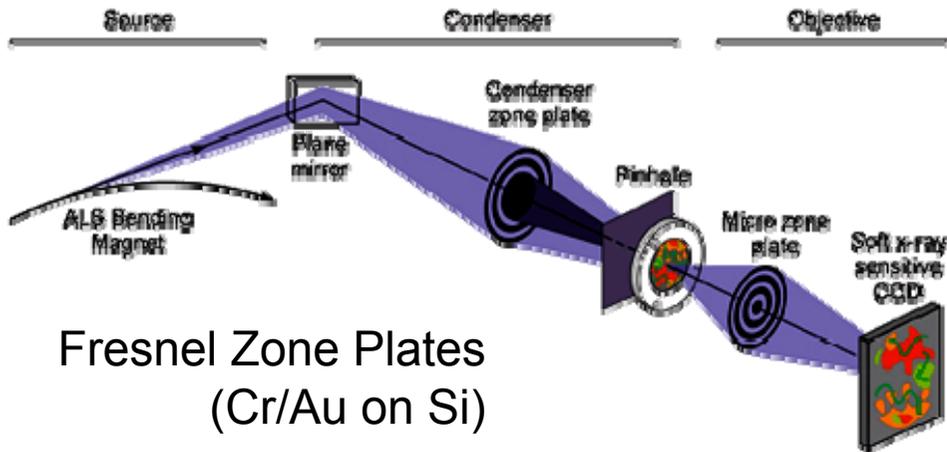
AC 80, pp. 6436-6442 (2008) by Dik *et al*



Patch of Grass (1887)



Weilun Chao *et al.* – NATURE 2005



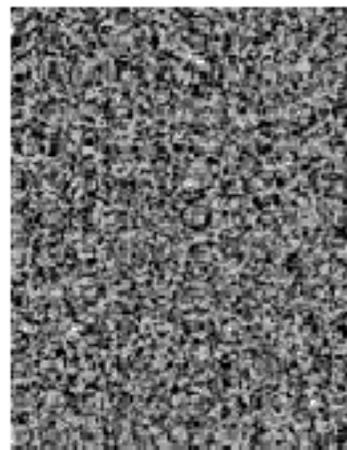
Advanced Light Source

Microscopía de rayos X blandos

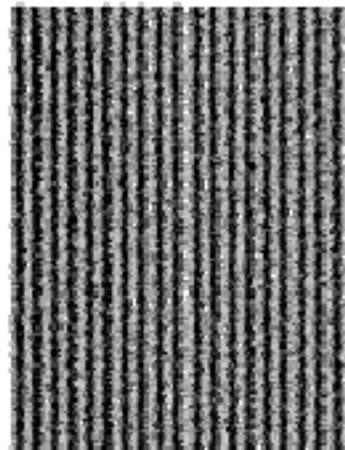


Resolución espacial nanométrica (15nm)

Images of test object with a 16.1-nm lines and spaces



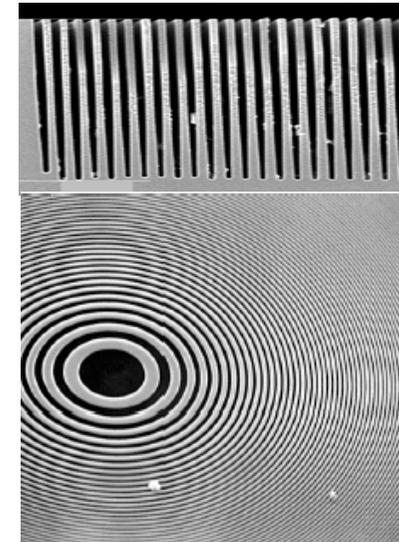
26-nm micro zone plate



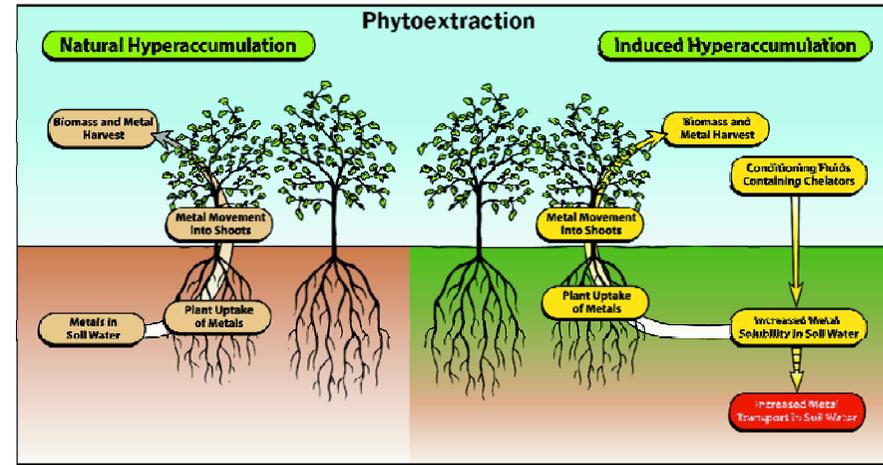
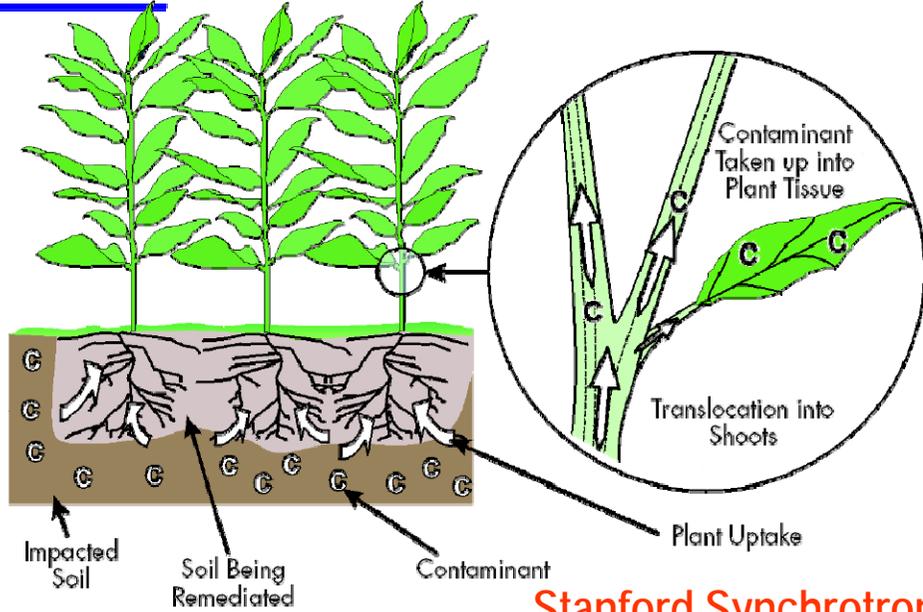
15-nm micro zone plate

$$\delta_m = 1.22 \frac{\Delta r}{m}$$

$$f_m = \frac{D \Delta r}{m \lambda}$$



Om Parkash Dhankher *et al.* – NATURE Biotechnology 2002

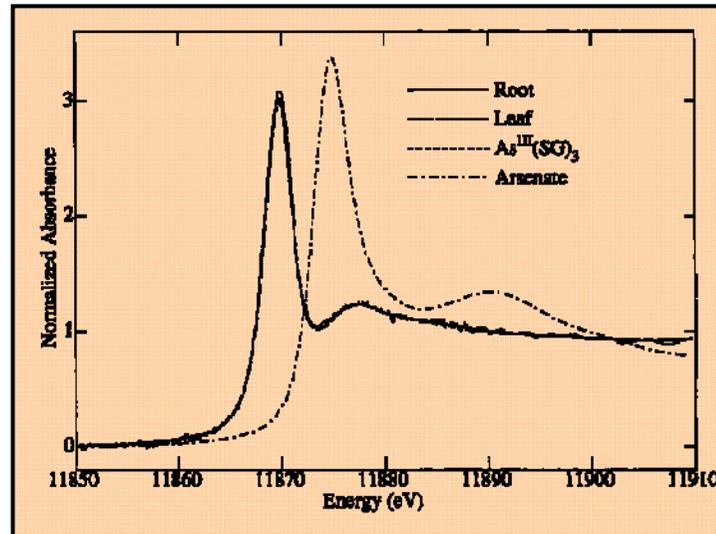


Stanford Synchrotron Radiation Laboratory

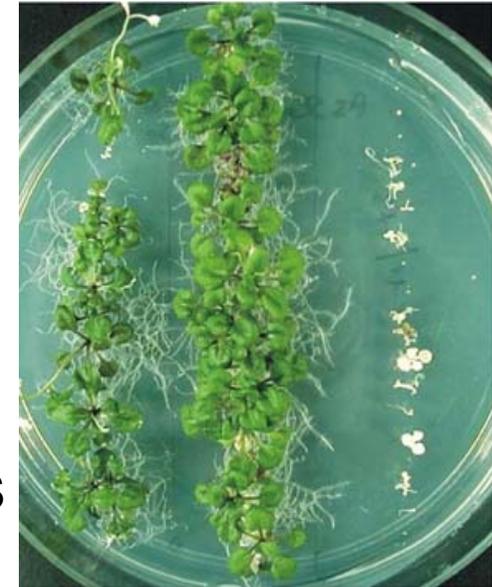
Planta transgénica
"mustard plant"

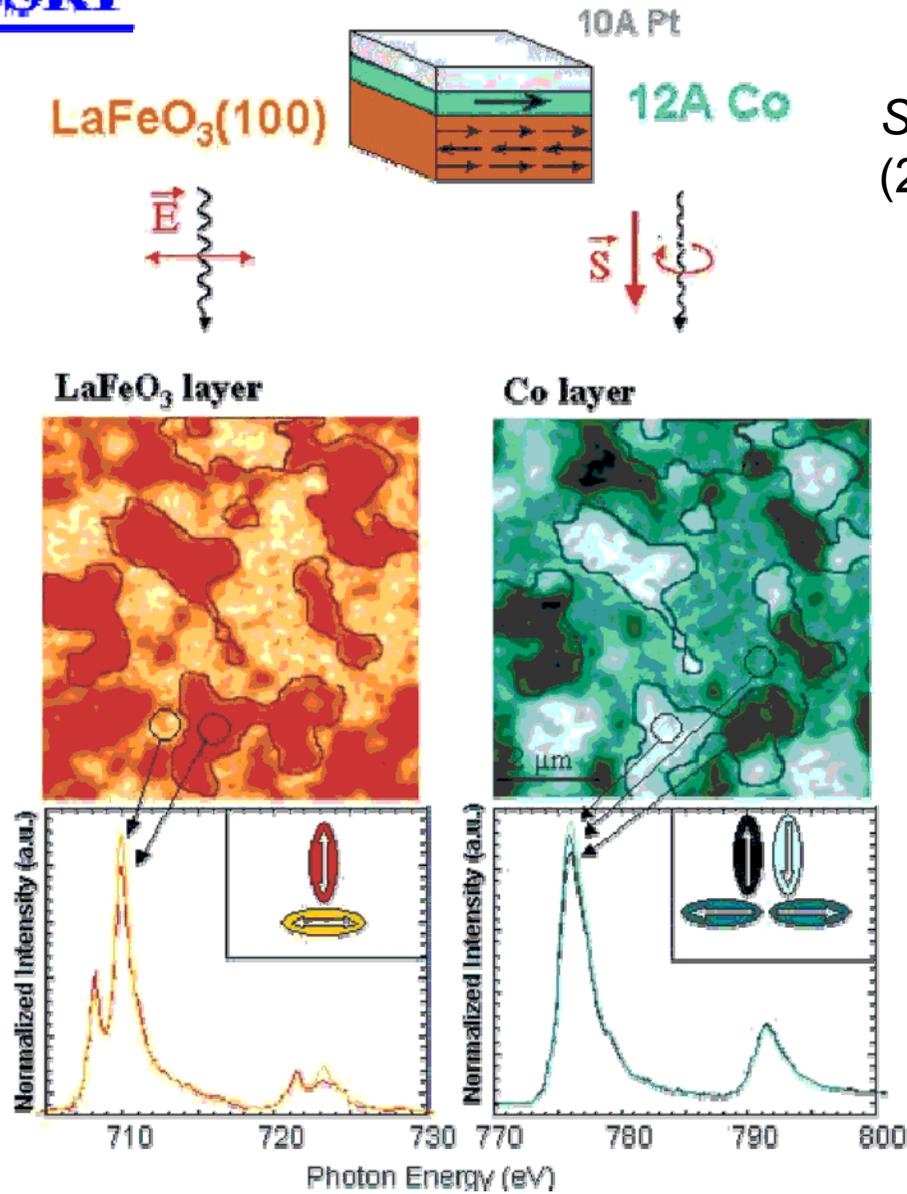


Acumula de 2–4 veces más de As en hojas y tallo



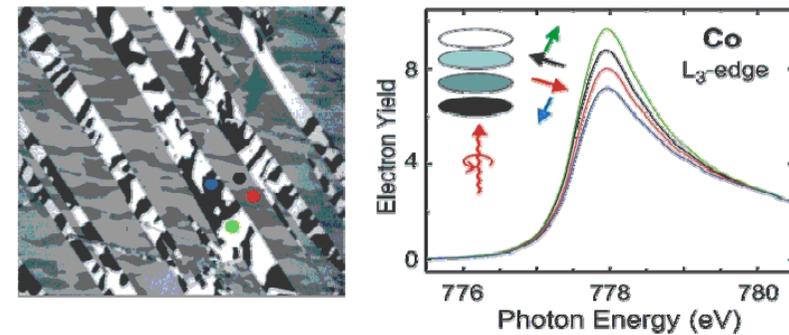
XANES
EXAFS





Science Vol. 306. no. 5696, pp. 686-687
(2004) by K.M. Kemner et al

XMCD Spectro-Microscopy





Bibliografía:

- “Elements of Modern X-ray Physics” – *Jens Als-Nielsen, Des McMorrow*
- “X-Ray Microscopy” – *Graeme R. Morrison*
- “Lectures on the x-ray microscope” – *W.C. Nixon*
- “Microscopic X-Ray Fluorescence Analysis” – *Koen H.A. Janssens,
Freddy C. V. Adams*
- “Handbook of X-Ray Spectrometry Revised and Expanded” (Practical Spectroscopy, V. 29) – *R. Van Grieken, Andrzej A. Markowicz, Rene E. Van Grieken*
- “X-Ray Microscopy in Biology and Medicine” – *Kunjo Shinohara Keiji Yada*
- “X-Ray Microscopy: Instrumentation and Biological Applications” – *Ping-Chin Cheng,
Gwo-Jen Jan*