Getting Started in Cryo-EM

△ Why electrons?

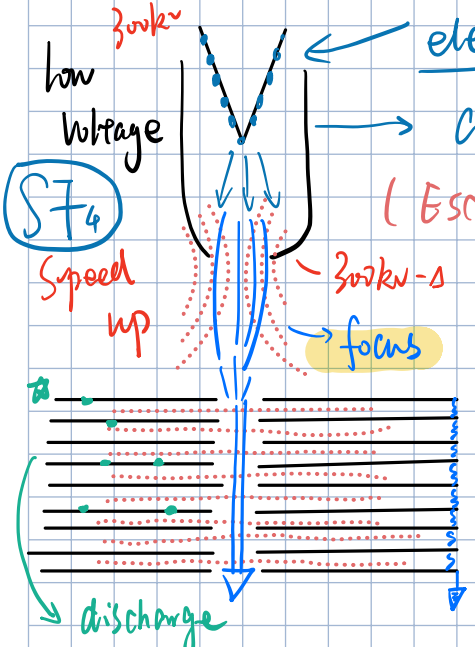
	Advantages ☺	Disadvantages ☹
Visible light	Not Damaging Easily focused Eye detector	Long wave length → (~ 400nm) ☹
X-rays	Small wavelength Good Penetration	Hard to focus Damage Sample
Electrons	great wavelength (pm) Be focused	Poor Penetration Damage Sample
Neutrons	great wavelength (pm) low damage	Produce? focus?

⇒ most effecient!
Find its niche!

△ Computatinal Tech

- Structrally and spatially explicit cell modeling.
- Molecular dynamics stimulations

Electron Guns: (a bent wire)



→ collide with neighbors & receive enough energy to escape
(Escape the function)

△ Contained in Vacuum
△ Vapor (etc.) → Stalactites
Clean regularly (keep Volt)

a series of disks
"accelerator stack"
→ graded drop in potential!

Result: 76% speed of light / $\lambda = 2pm$ ← Smaller detail!

!! Safety !!

☆ The can is insulated By SF_6 to keep enough voltage (in a tank)

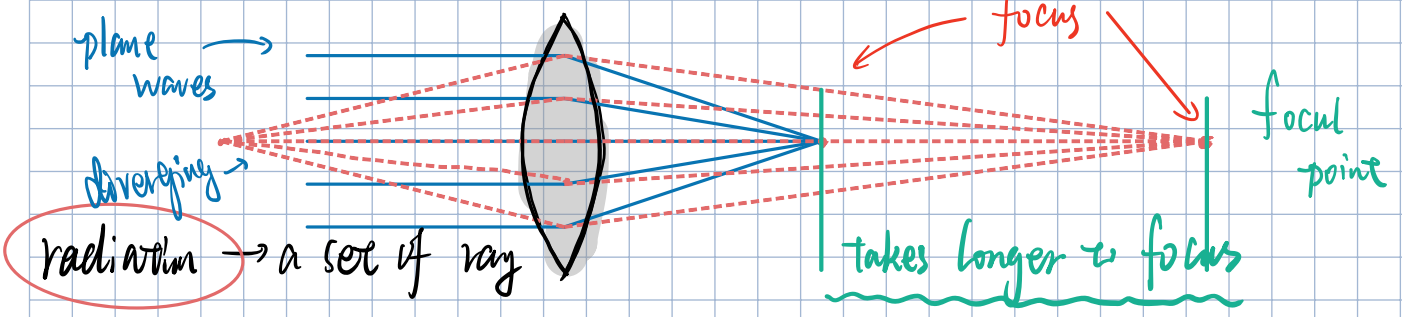
Two Types of Coherence

- **Spacial coherence:** Do all the electrons come from the same direction. (blur)
- **Temporal coherence:** Do they all have the same speed? (defocus)

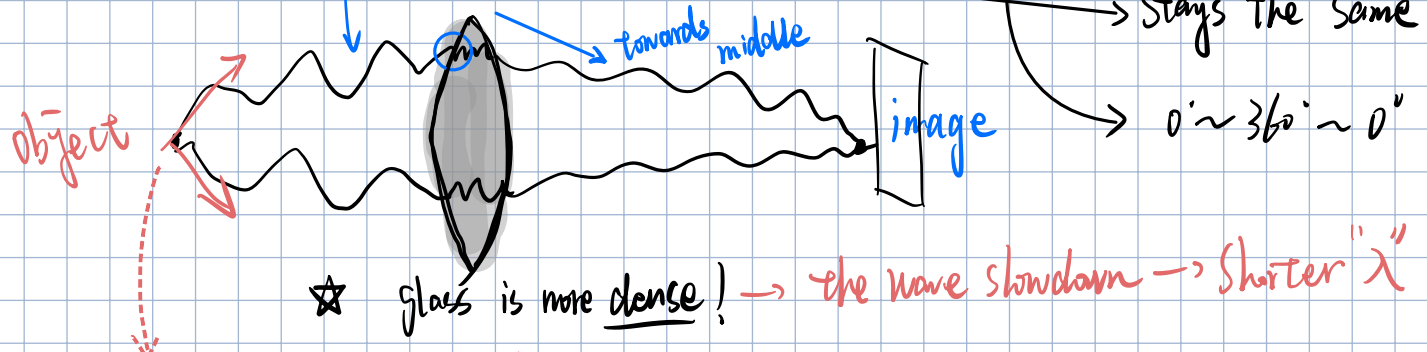
Three most common types of filaments:
1. Tungsten filament (cheap, quick to replace)
2. Lanthanum hexaboride (LaB_6) crystal (Sharp → Spacial / Speed ✓)
3. Field emission Gun (pull out e^-) (Greater Coherence) Cold FEG, Warm FEG (current ↑)

↓ de over time (fineness ↓) → Find the best warmup temprature

EM Lens → bends radiation to focus it



as waves to be exactly (amplitude & phase)

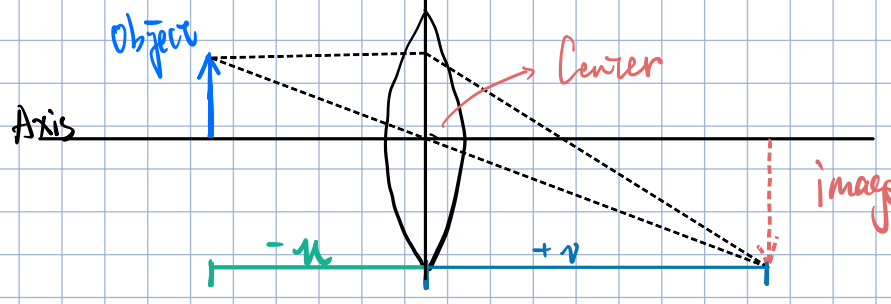


☆ glass is more dense! → the wave slowdown → Shorter "λ"

their optical path length are equal!

Put a detector (film) → to collect most photons

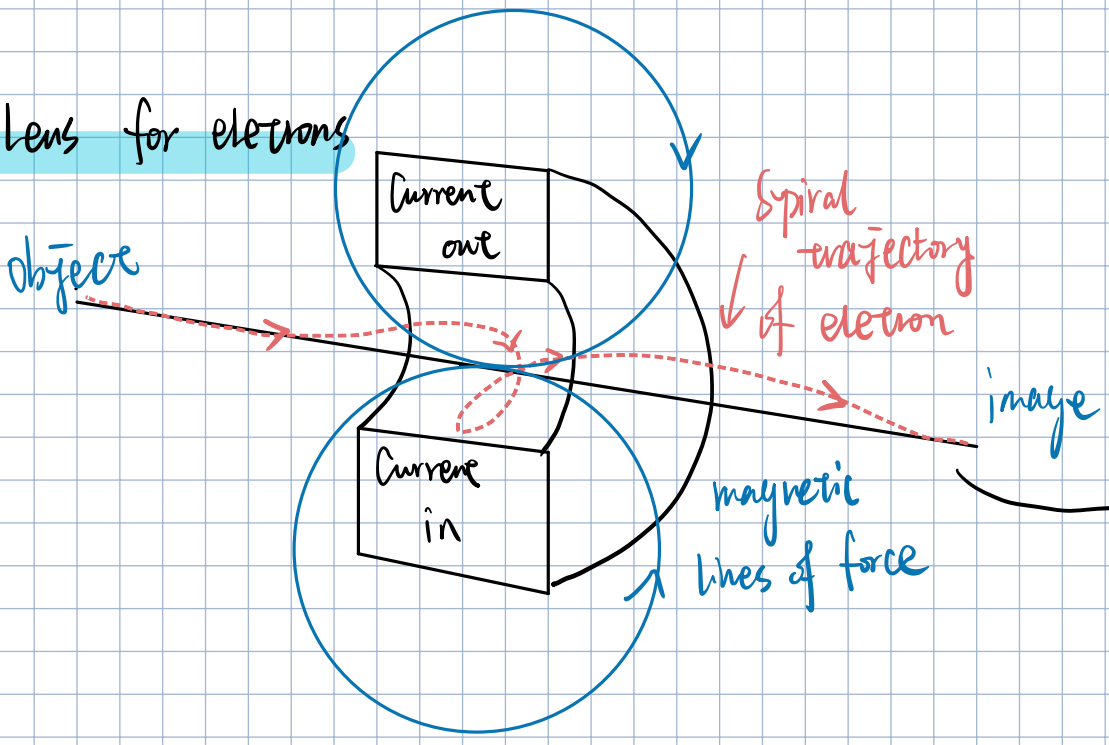
Optical Lens



$$\begin{cases} M = v/u & \text{(magnification)} \\ \frac{1}{f} = \frac{1}{u} + \frac{1}{v} & \text{(thin lens eqn)} \end{cases}$$

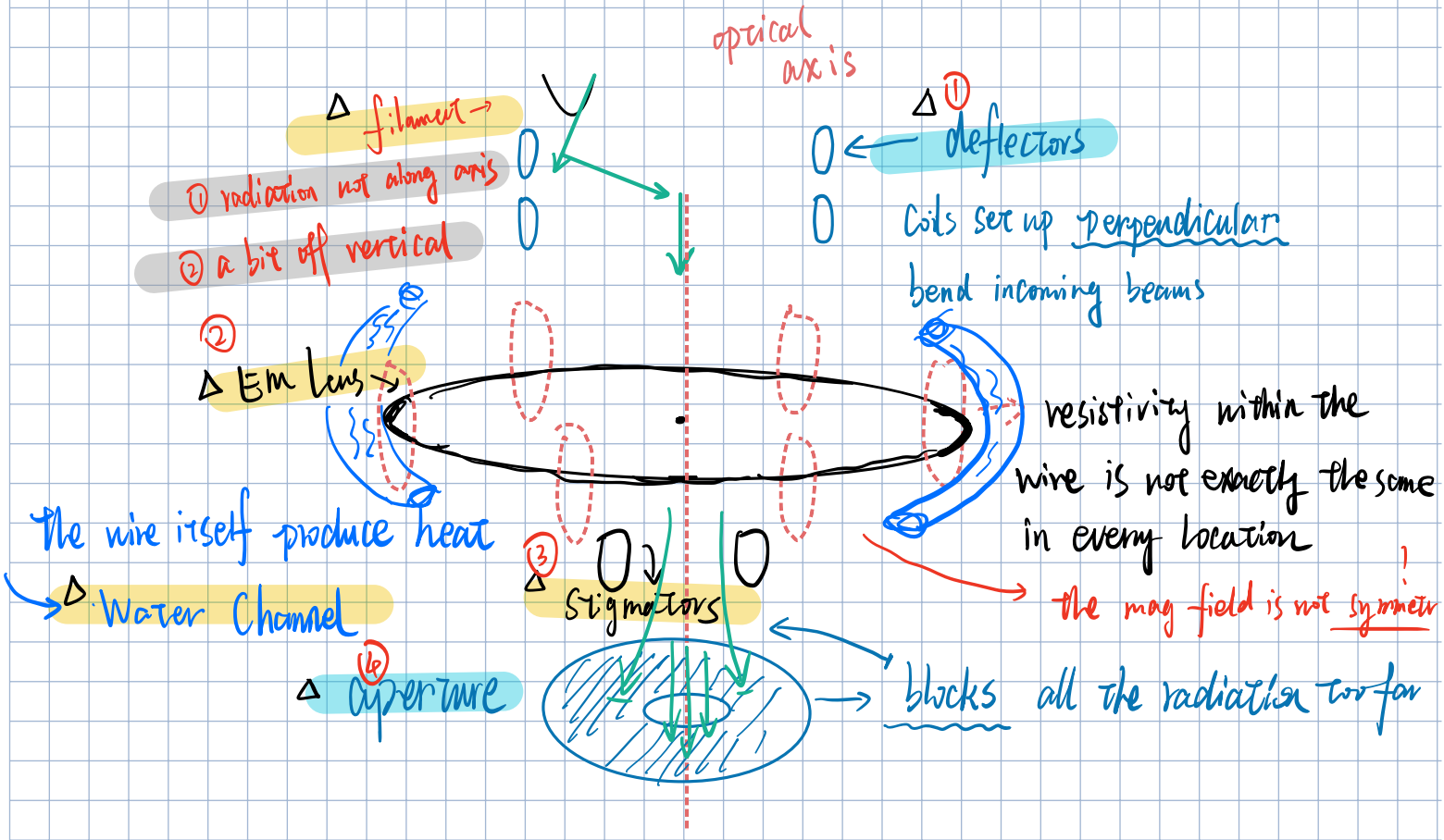
☆ an electron could be thought of as a wave ⇒ EM?

Lens for electrons



→ the spiral will creats the image to stir

EM lens system



Stigmatic → the magfield is stronger → the electrons focus stronger!

Stigmators → Compensate for the asymmetric produced by lens itself

The Column, knobs and names

electron microscope is a Compound microscope (1 ~ 5,000,000 M)

Gun → Condensor system → Objective system → Projector Lens System

align a microscope?

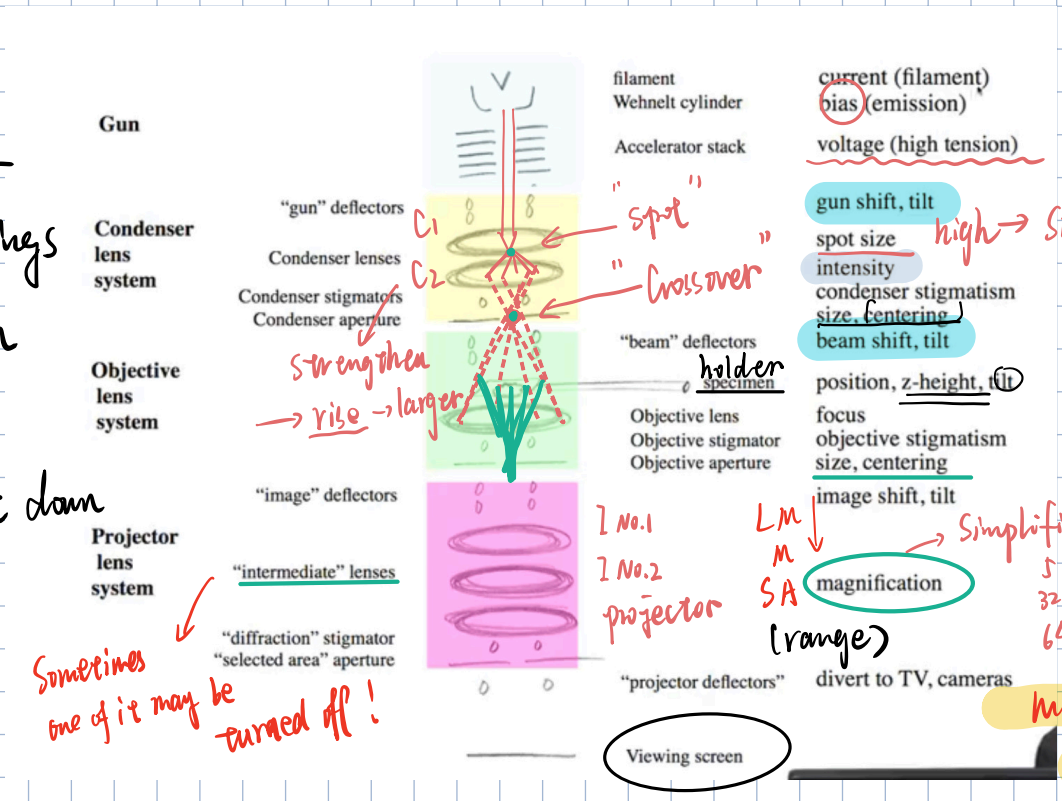
different settings to take emission

shift / tilt

↓

Come right down

Deflector settings!



Sometimes one of it may be turned off!

Less bright (dim)

high → Small Spot

more coherent

Currents one setup

minimize the rotation!

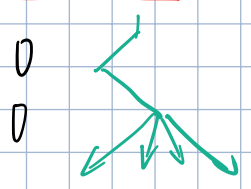
① Shape of the tip can change over time

② Temprature change subtly ⇒

set of parameters

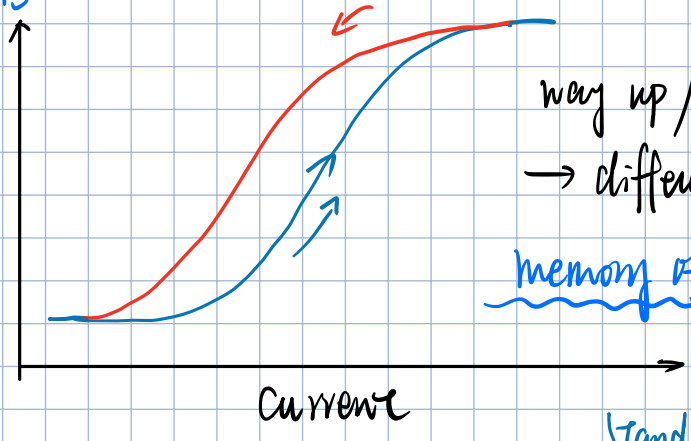
align the pivot points

pivot point alignment



"tilt"

hysteresis?



way up / down → different mag fields?

memory of metal!

5K

↓

18K

↓

32K

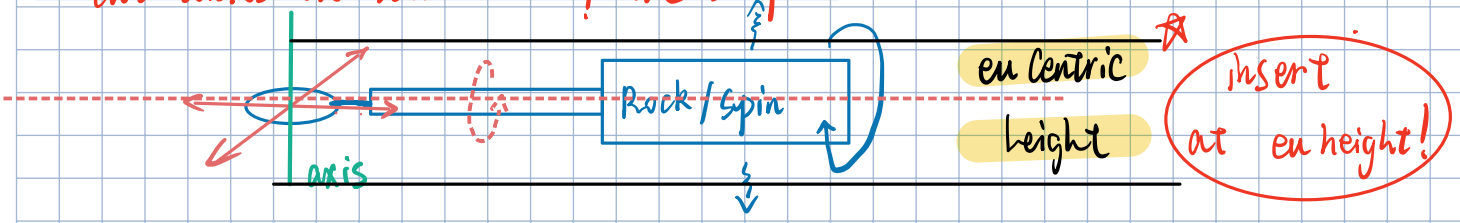
Conditions are not right!

Standerize the result ← normalize

* pole pieces gap { smaller → higher resolution ⇒ water in column
bigger → rotate the sample (cryobox) freeze on sample !

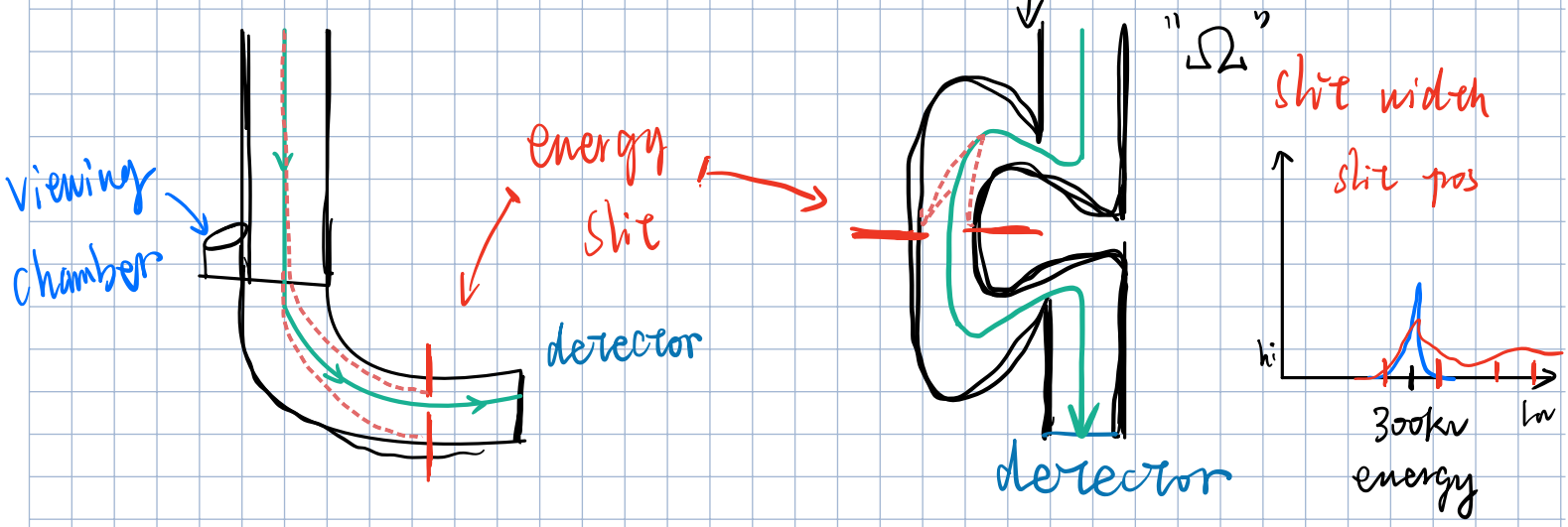
(as a result)

two disks on both sides of the sample



energy filters

{ post-column filters
in-column (omega) filters



Electron detectors

{ Flourescent screens
TVs (live) → large image / high resolution ∴ time consuming
Photographic film (digitized)
CCDs (scintillator → fiber optic → CCD → amplifier)
"Direct" detectors CMOS ∵ read-out quickly !

May scatter too far → even come back → two/more spot of one electron !

Single e⁻ → uncertain number of photon → uncertain brightness.