MICRO-561

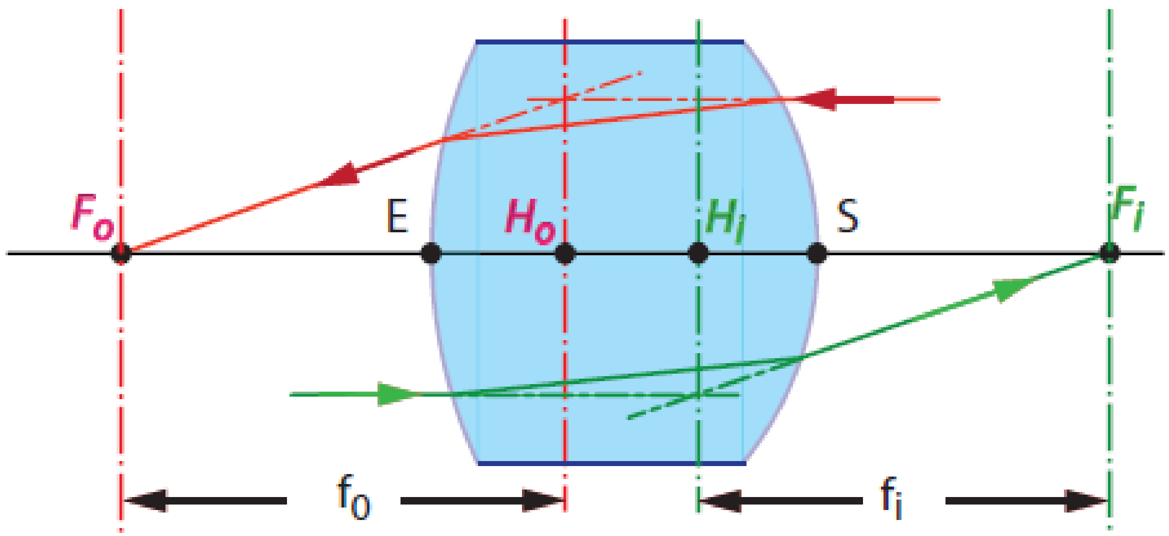
Fundamentals of Biomicroscopy

Syllabus (tentative)

Lecture 1	Introduction & Ray Optics-1
Lecture 2	Ray Optics-2 & Matrix Optics-1
Lecture 3	Matrix Optics-2
Lecture 4	Matrix Optics-3 & Microscopy Design-1
Lecture 5	Microscopy Design-2
Lecture 6	Microscopy Design-3
Lecture 7	Resolution-1
Lecture 8	Resolution-2
Lecture 9	Resolution-3 & Contrast
Lecture 10	Fluorescence-1
Lecture 11	Fluorescence-2
Lecture 12	Fluorescence-3, Sources, Filters
Lecture 13	Detectors
Lecture 14	Bio-application Examples

Review: Principal points and principal planes

- **Vertex** E, S — Intersection point of the first/last interface (surface) system with the optical axis.

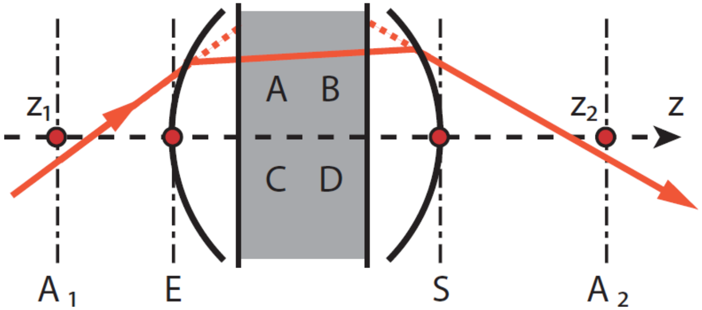


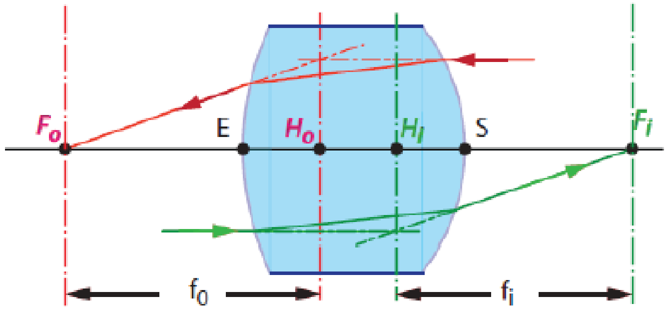
<u>Ho & Hi:</u>

- **Principal Planes** are located where the "***apparent***" ray bending takes place.

- **Principal Points** are located at the intersection of the principal planes with the optical axis. (also called nodal points)

Summary: Determine Cardinal Points & Planes with [ABCD]





- This table is valid for a **generalized** optical system between E&S and not limited to the thick lens example that we studied in previous lecture (and shown on the right).

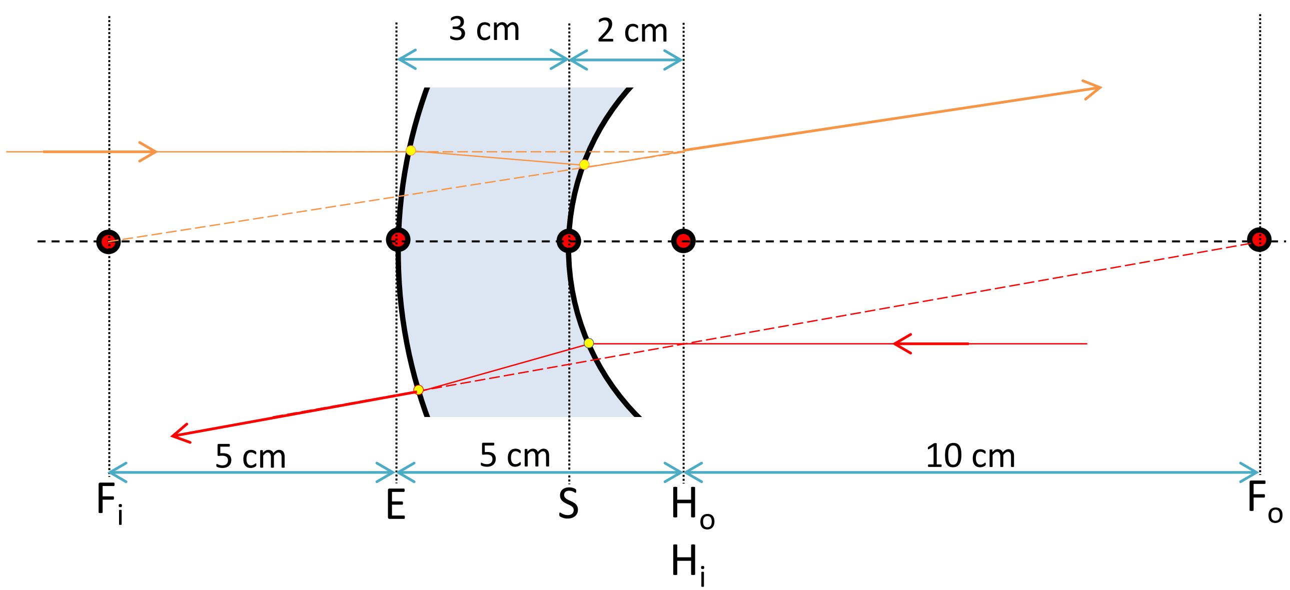
Distances	Notation	Directed Distances	ABCD elements
Object Focal Point	F_o	$\overline{EF_o}$	D/C
Object Focal Length	f_o	$\overline{H_oF_o}$	$1/C$
Object Principle Point	H_o	$\overline{EH_o}$	$(D-1)/C$
Image Focal Point	F_i	$\overline{SF_i}$	$-A/C$
Image Focal Length	f_i	$\overline{H_iF_i}$	$-1/C$
Image Principle Point	H_i	$\overline{SH_i}$	$(1-A)/C$

From last lecture: Example-1 thick lens with [ABCD]

Lens parameters	Num. value
1st interface curvature (R_1)	+5 cm
2nd interface curvature (R_2)	+2 cm
Central thickness, e	3 cm
Lens index (glass)	1.5

$$T(ES) = \begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} 0.8 & 2 \\ 0.1 & 1.5 \end{bmatrix}$$

Distances	Notation	Directed Distances	ABCD elements	Calculated Value
Object Focal Point	F_o	$\overline{EF_o}$	D/C	+15 cm
Object Focal Length	f_o	$\overline{H_oF_o}$	$1/C$	+10 cm
Object Principle Point	H_o	$\overline{EH_o}$	$(D-1)/C$	+5 cm
Image Focal Point	F_i	$\overline{SF_i}$	$-A/C$	-8 cm
Image Focal Length	f_i	$\overline{H_iF_i}$	$-1/C$	-10 cm
Image Principle Point	H_i	$\overline{SH_i}$	$(1-A)/C$	+2 cm



Cardinal plane(s)

- ***Principal planes H_o, H_i***
- ***Not always inside the thick lens***
- ***The refraction happens at the virtual/fictious principal plane***
- ***Always magnification M = 1 between principal planes***
- ***Focal plane(s) F_o, F_i***
- ***Common point for rays parallel to axis***

How to trace rays through a thick lens?

The aim is to trace rays through a thick lens much like we did through a thin lens.
In other words, we seek to replace the thick lens by a paraxial lens that reproduces its characteristics

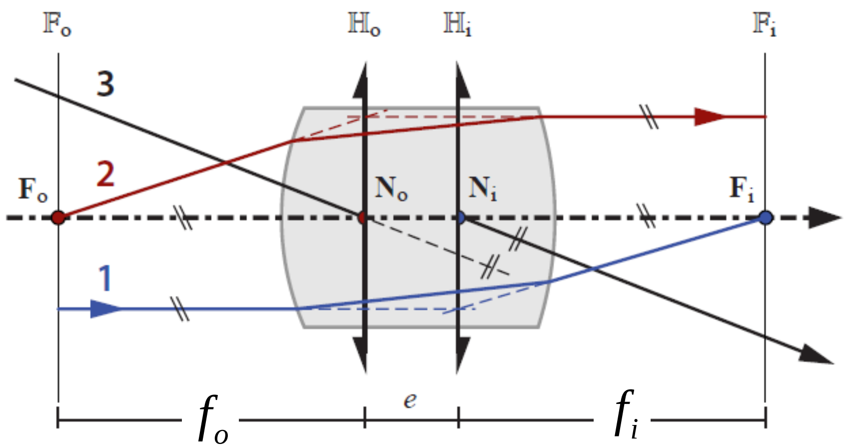


Illustration: Cardinal elements and paraxial lens model of a thick lens reproducing the lens' characteristics

Blue ray ➔ 1. An incident ray parallel to the optical axis intersects it in the image focal point $\mathbf{F}_i$. It appears to be deflected at the image principal plane $\mathbb{H}_i$. The focal length f_i is the air distance $\mathbb{H}_i \rightarrow \mathbb{F}_i$ and the image space appears to begin at $\mathbb{H}_i$.

Red ray ➔ 2. An incident ray from the object focus $\mathbf{F}_o$ exits parallel to the optical axis.[4] It appears to be deflected at the object principal plane $\mathbb{H}_o$. The focal length f_o is the air distance $\mathbb{F}_o \rightarrow \mathbb{H}_o$ and the object space appears to extend up to $\mathbb{H}_o$.

Black ray ➔ 3. An incident ray through the object nodal point $\mathbf{N}_o$ exits parallel to the incident ray but appears to stem from the image nodal point $\mathbf{N}_i$.

Link to ray tracing: Tracing of an arbitrary incident ray for a thick lens

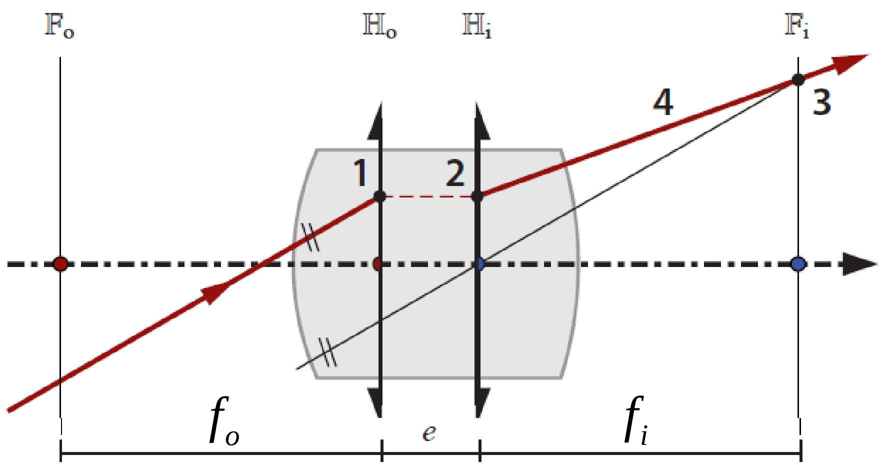


Illustration: Trace arbitrary rays through a thick lens

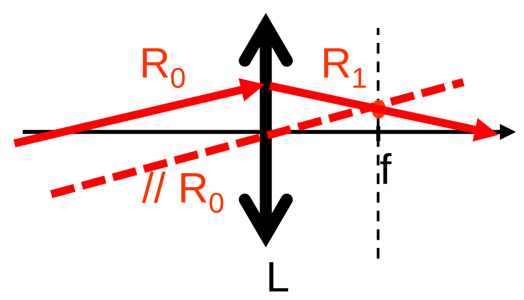
Remember from thin lens: Tracing an arbitrary ray

→ The ray goes through the intersection point of:

- a parallel «**helper**» ray (passing through the center of the lens, indicated by dashed red line)

and

- the vertical line at the focal plane of the lens (indicated by black dashed line)



Link to ray tracing:
Tracing of an arbitrary incident ray for a thick lens

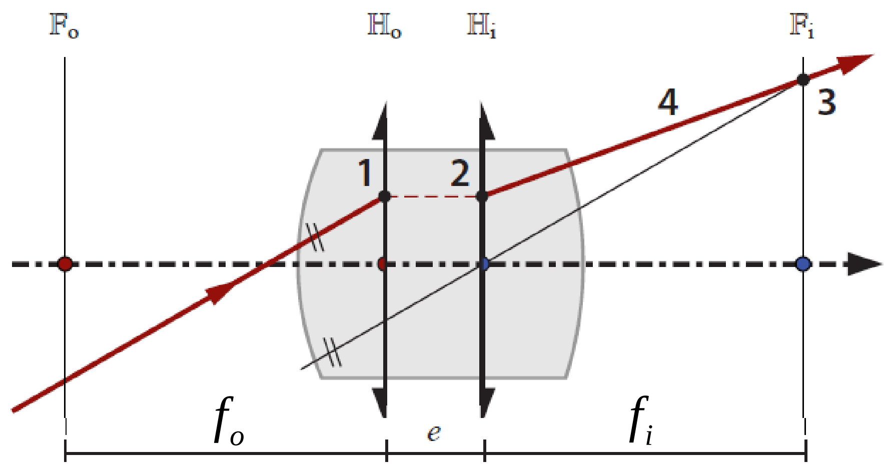


Illustration: Trace arbitrary rays through a thick lens

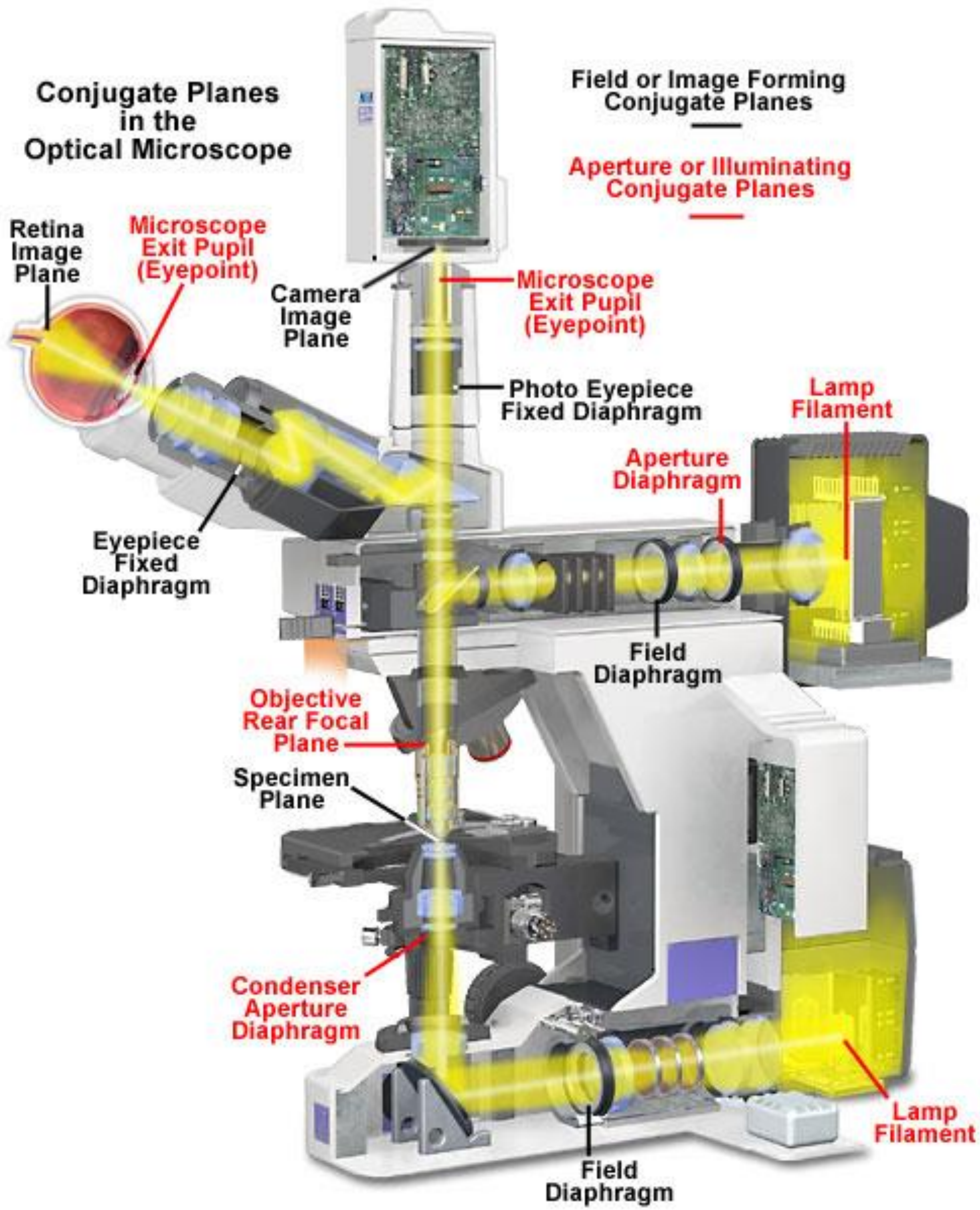
1. Intersect the incident ray with the object principal plane $\mathbb{H}_o$.

Outline

Optical Microscopy Design

- Spherical Aberration
- Lateral Limitations in Microscopy
- Aperture Stop
  - Numerical Aperture of a Microscope
  - Entrance Pupil
  - Exit Pupil
  - Marginal Rays
  - Chief Rays
- Field Stop
  - Field-of-View of a Microscope
  - Entrance Window
  - Exit Window

Microscopy Design



A microscope consists of a series of optical elements:

- Lenses
- Diaphragms
- Filters
- Polarizers …etc

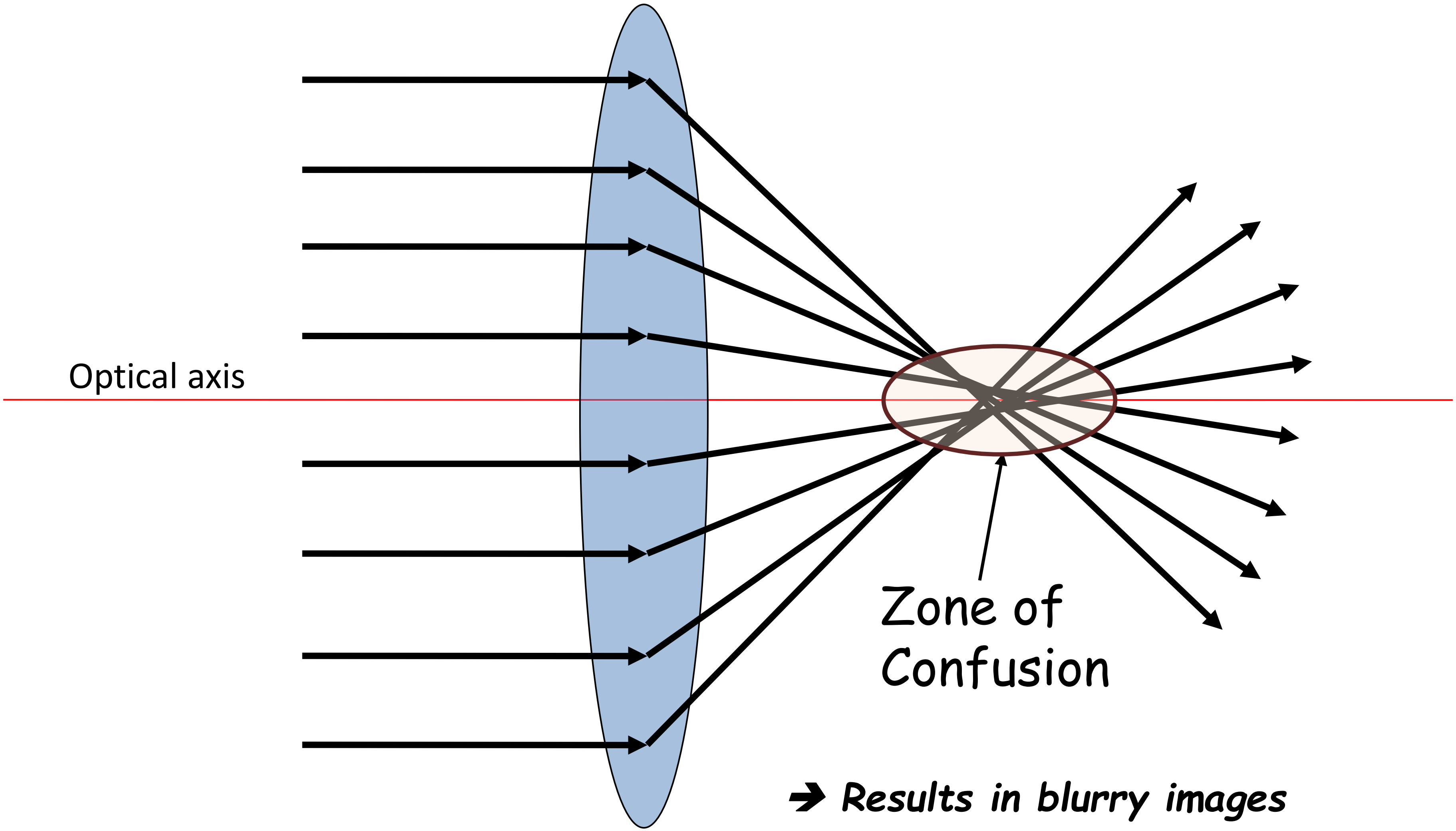
Major Optical Aberrations in Microscopy

- The major six aberrations are:
  - Spherical aberration
  - Chromatic aberration
  - Coma
  - Astigmatism
  - Curvature of field
  - Distortion

- Lenses are associated with many of these ***aberrations*** that can distort the image in various ways.

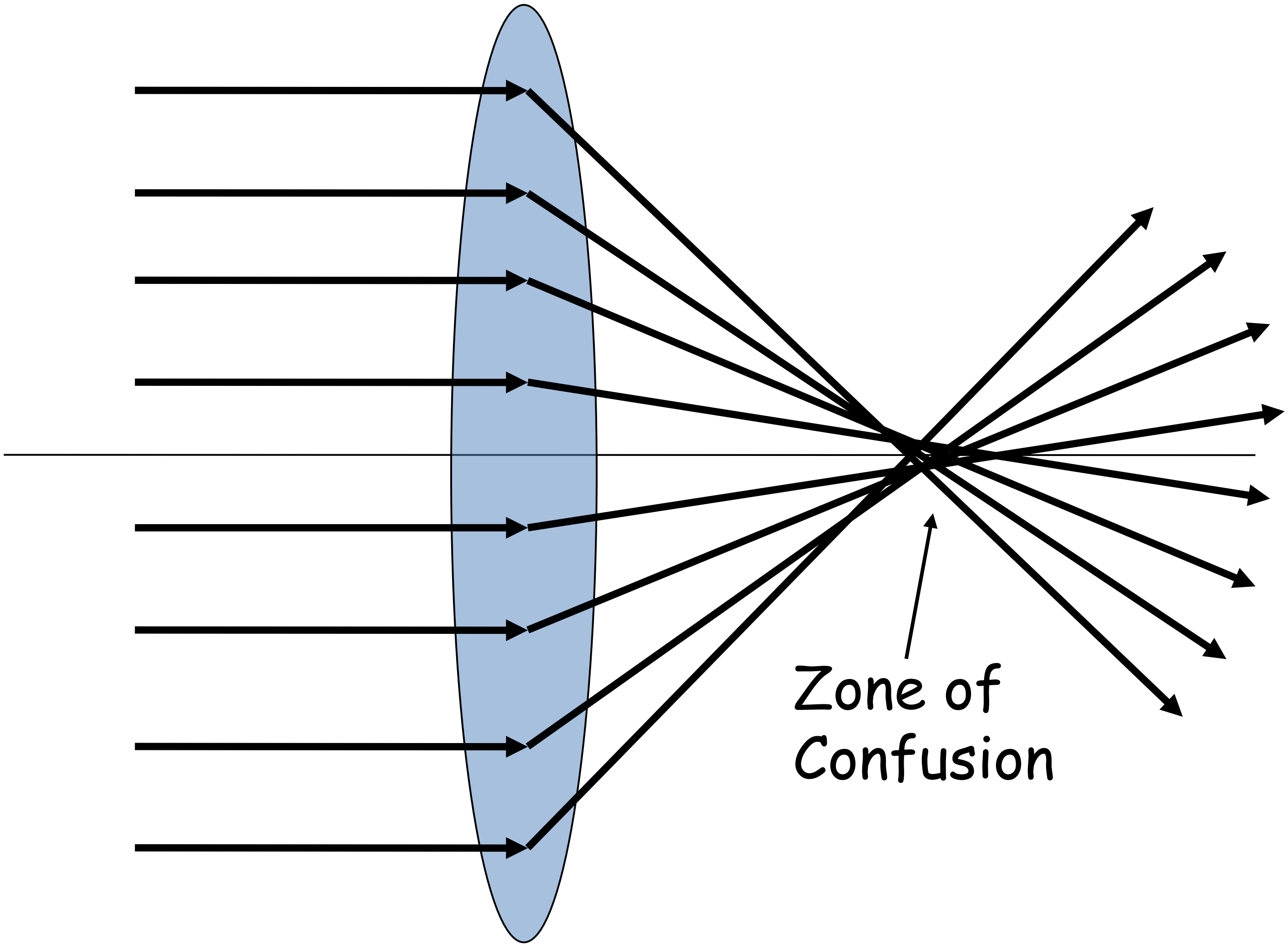
Spherical Aberration

Incident rays parallel to the optical axis and reaching the center and the periphery of the lens are focused at different locations.



➔ ***Results in blurry images***

Spherical Aberration

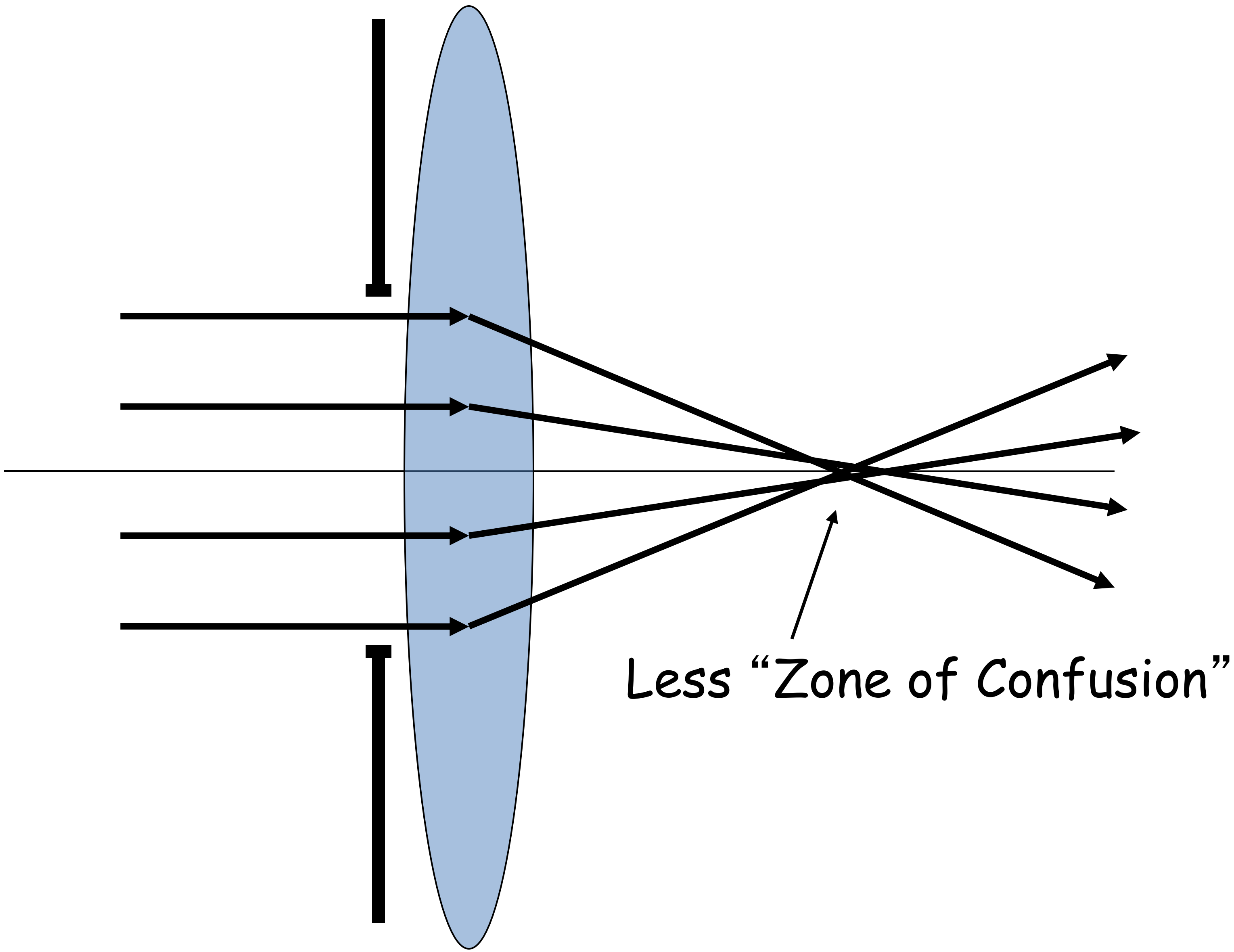


Spherical aberration gets worse near the lens edges:

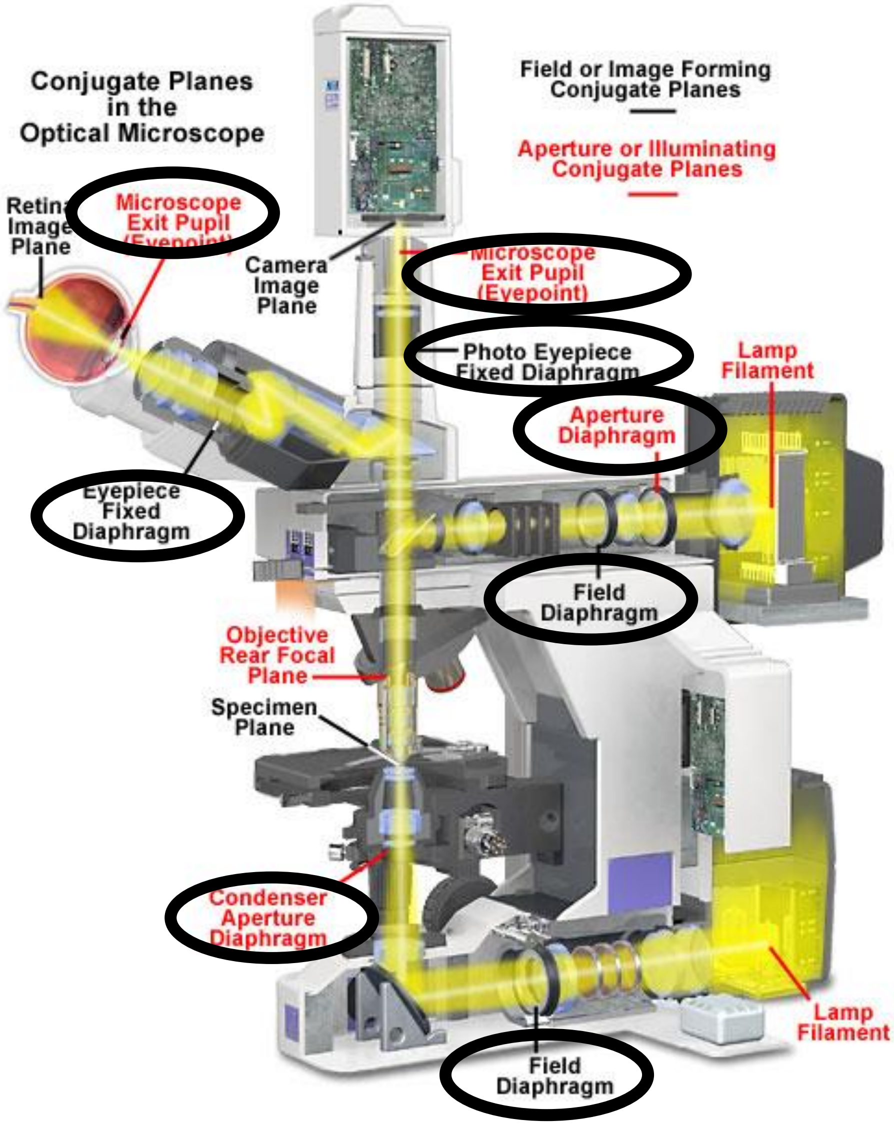
→ rays near the lens edge bent more

One potential solution: Limit the range of lens openning

Spherical aberration can be reduced by using an "aperture"



Lateral Limitations in Optical System



- Aperture Stop/Diaphragm
- Entrance Pupil
- Exit Pupil

- Field Stop/Diaphragm
- Entrance Window
- Exit Window

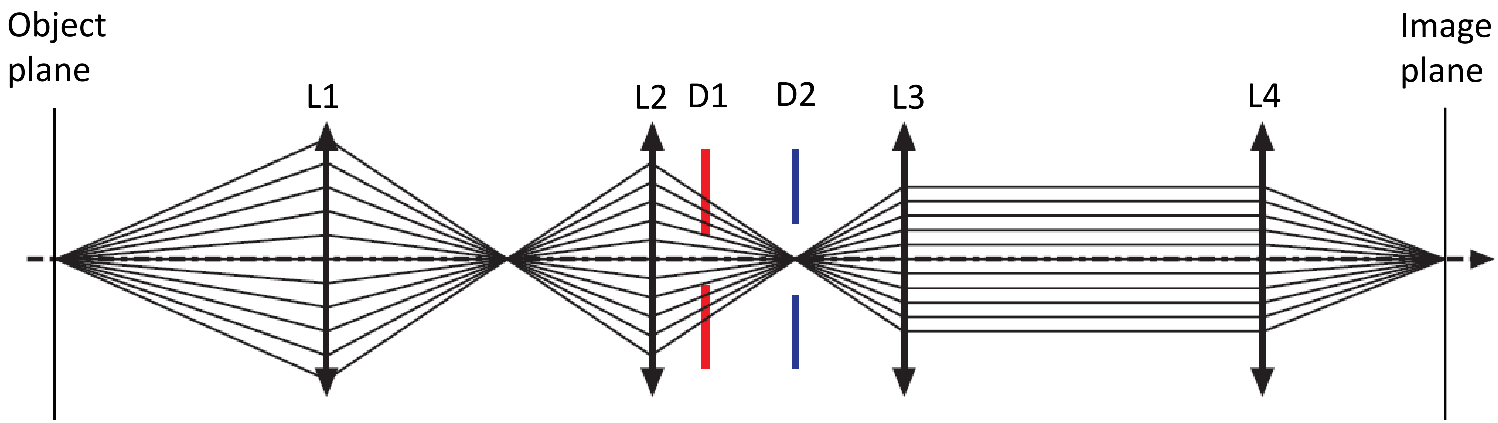
Lateral Limitations in Optical Systems

- Due to finite physical dimensions, every optical component has a limited lateral extent called **clear diameter**.
- In particular, every spherical interface has a finite diameter limited by the radius of curvature.
- Besides these constraints, an optical system usually contains **dedicated diaphragms** that provide lateral limitations to the heights of rays at well-defined positions.

➔ **These dedicated diaphragms typically serve as aperture stop and field stop** and enforce unique properties of the optical system such as numerical aperture, field size, perspective, telecentricity, stray light suppression ...

Lateral Limitations in Optical Systems

A



Consider the example shown above in which rays that start at an axial point in the object plane are blocked by the lateral limitation of:

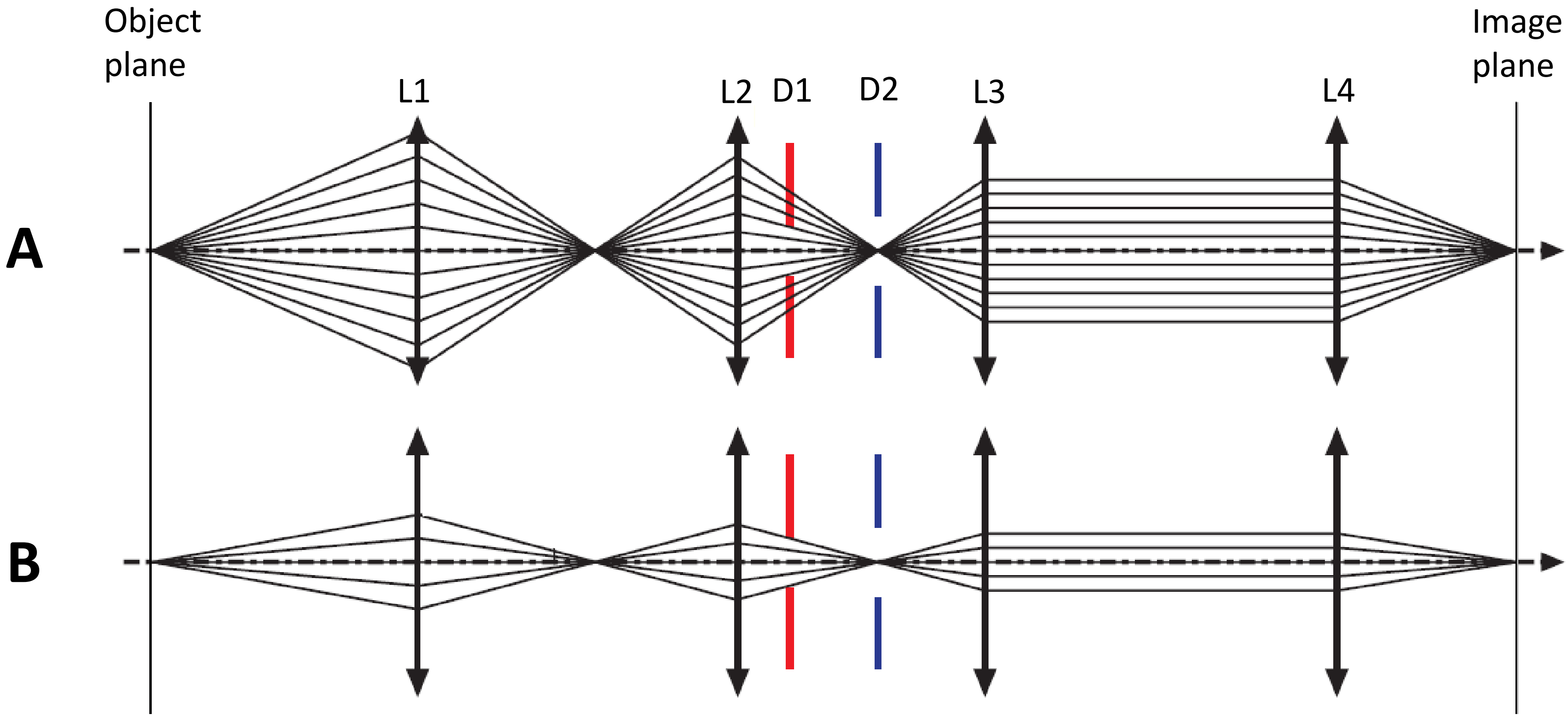
Four lenses: L_1, L_2, L_3, L_4

and

Two diaphragms: D_1, D_2

- **"The most limiting optical element" is called "aperture stop".**
- **In this example, which element is the most limiting optical element (i.e. aperture stop)?**

Lateral Limitations in Optical Systems



A –

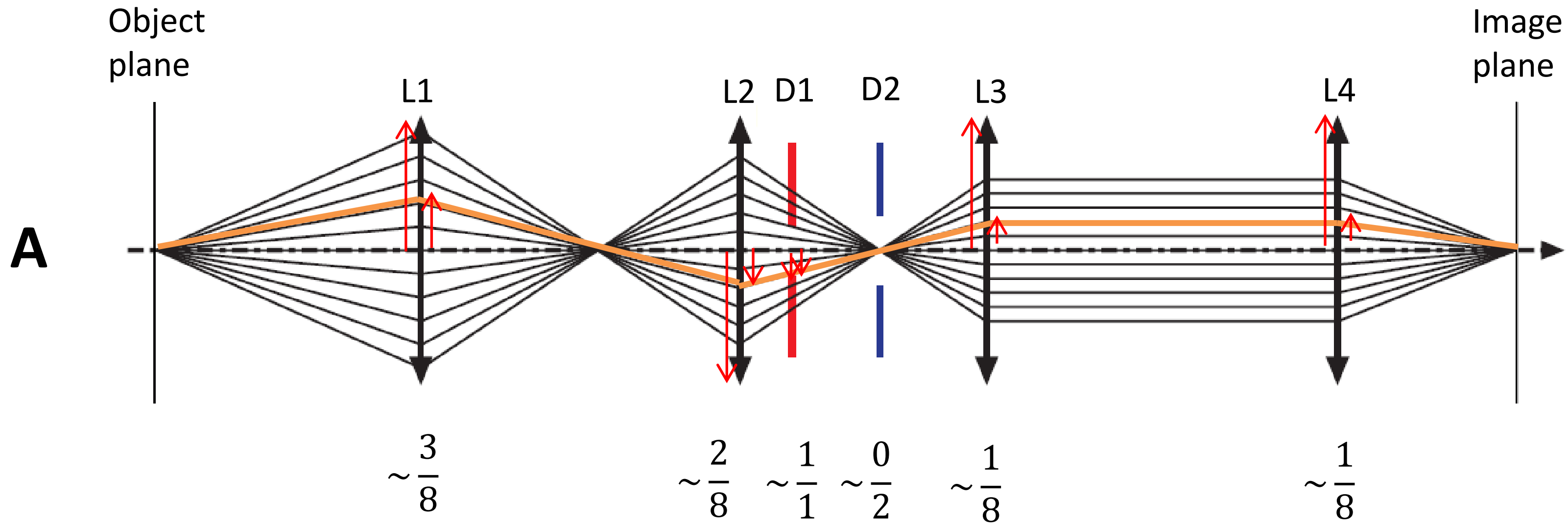
- In this example, shown rays are blocked by the lateral limitation of
  lenses L_1, L_2, L_3, L_4
  and
  diaphragms D_1, D_2

B –

- Diaphragm **D_1** is the most limiting element:
  ➔ In this example, aperture stop is **D_1**

"Aperture stop" may also called "aperture" or "stop"

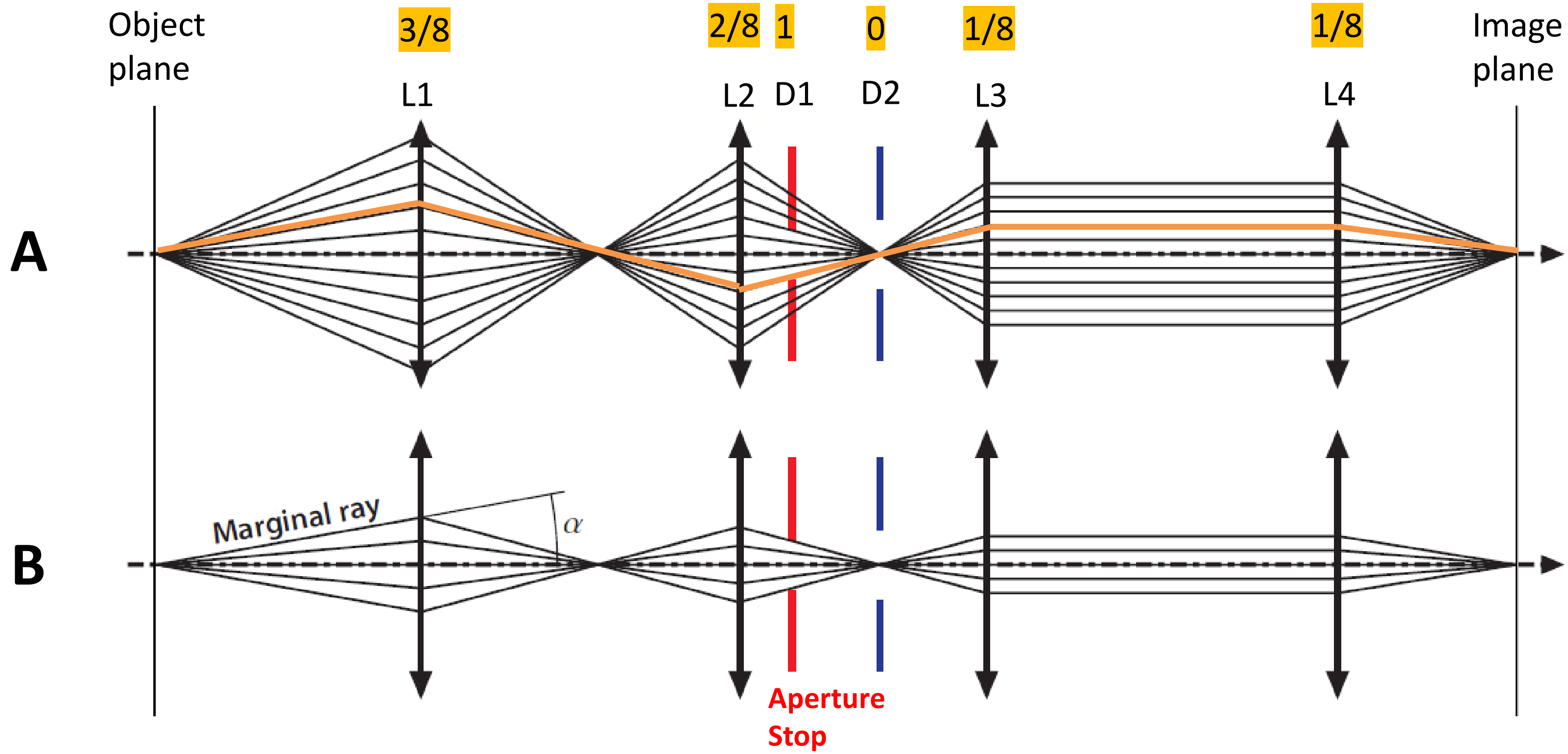
General procedure: how to find the aperture stop?



- In order to find the most limiting element for the diameter of the axial beam, **trace an arbitrary (marginal) ray** starting from the axial object point (as shown in A).
- For each element, **find the ratio** of the (marginal) ray height to the clear height.

The numbers are approximated for illustration purposes

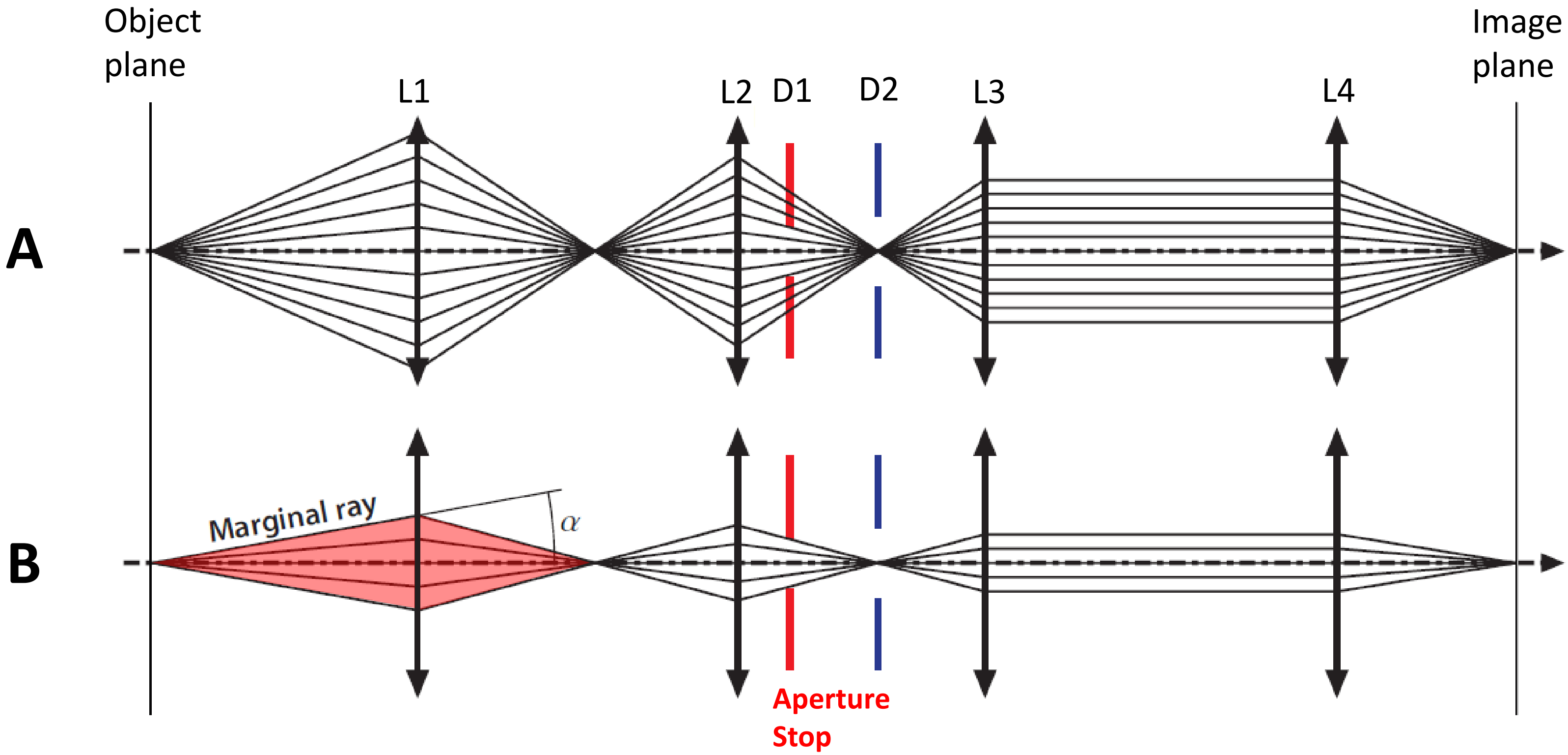
General procedure: how to find the aperture stop?



- In order to find the most limiting element for the diameter of the axial beam, **trace an arbitrary (marginal) ray** starting from the axial object point (as shown in A).
- For each element, **find the ratio** of the (marginal) ray height to the clear height.
- The element with the largest ratio limits the diameter of the ray bundle transmitted from the axial object point to the axial image point is the **aperture stop**. ➔D1 is "1"
- As shown in (B), the first diagram (D1) is the aperture stop.

The numbers are approximated for illustration purposes

Aperture stop determines the maximum opening angle of conical ray bundle



- The **numerical aperture** (NA) of the system is determined by the **angle between the two marginal rays passing through the two edges of the Aperture Stop**.
- The cone semi-angle α defines the numerical aperture **(NA)** of the optical system

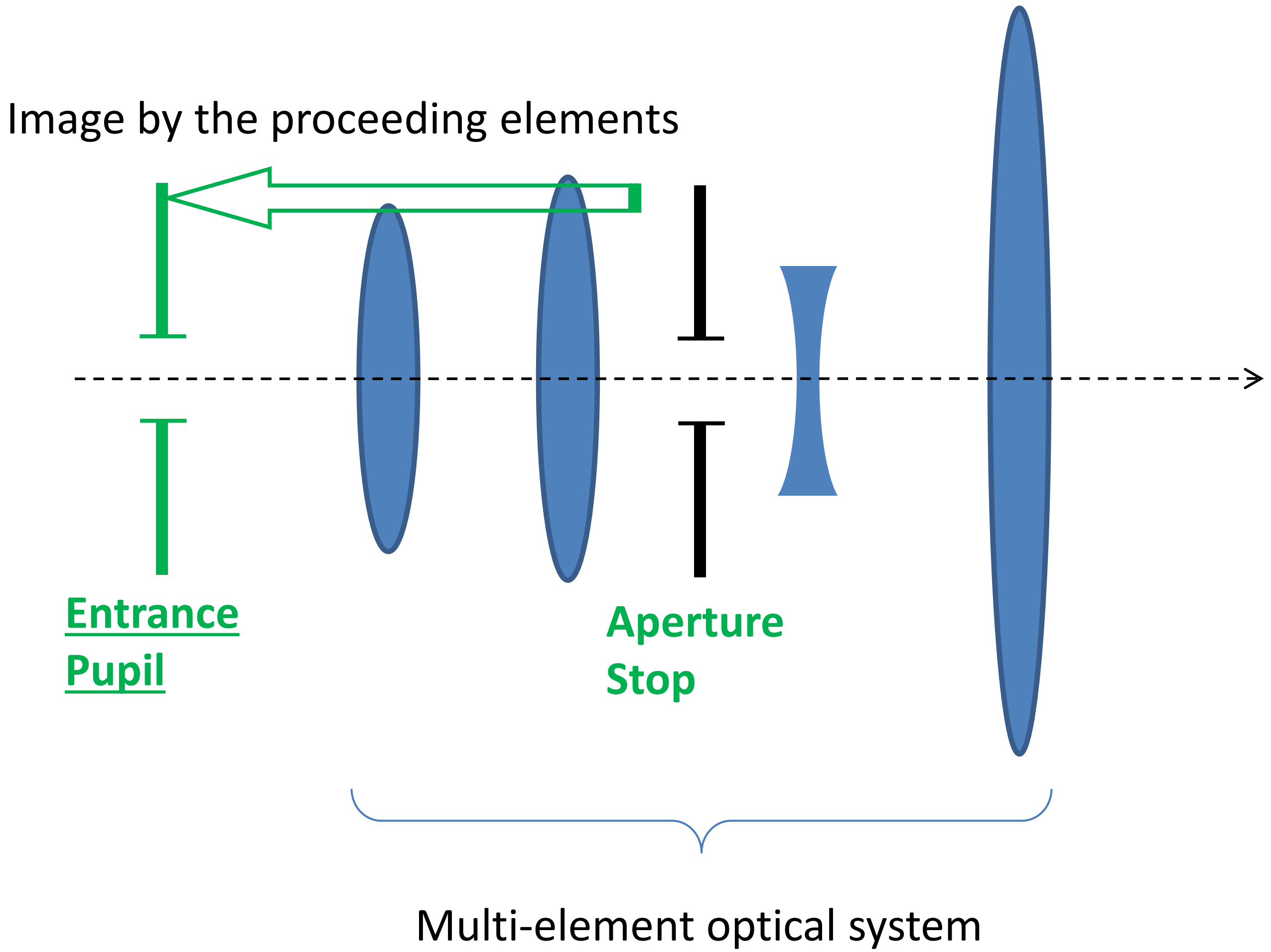
$$NA = n \times sin\alpha$$

➔ As we will see in in next lectures, NA will influence the resolution of microscope…

Entrance Pupil (E_nP)

Once we find the aperture stop, next we can determine entrance pupil (E_nP):

Entrance Pupil is defined as the image of the aperture stop by all the lenses preceding it (i.e. to the left of AS – as if light travels from left to right)

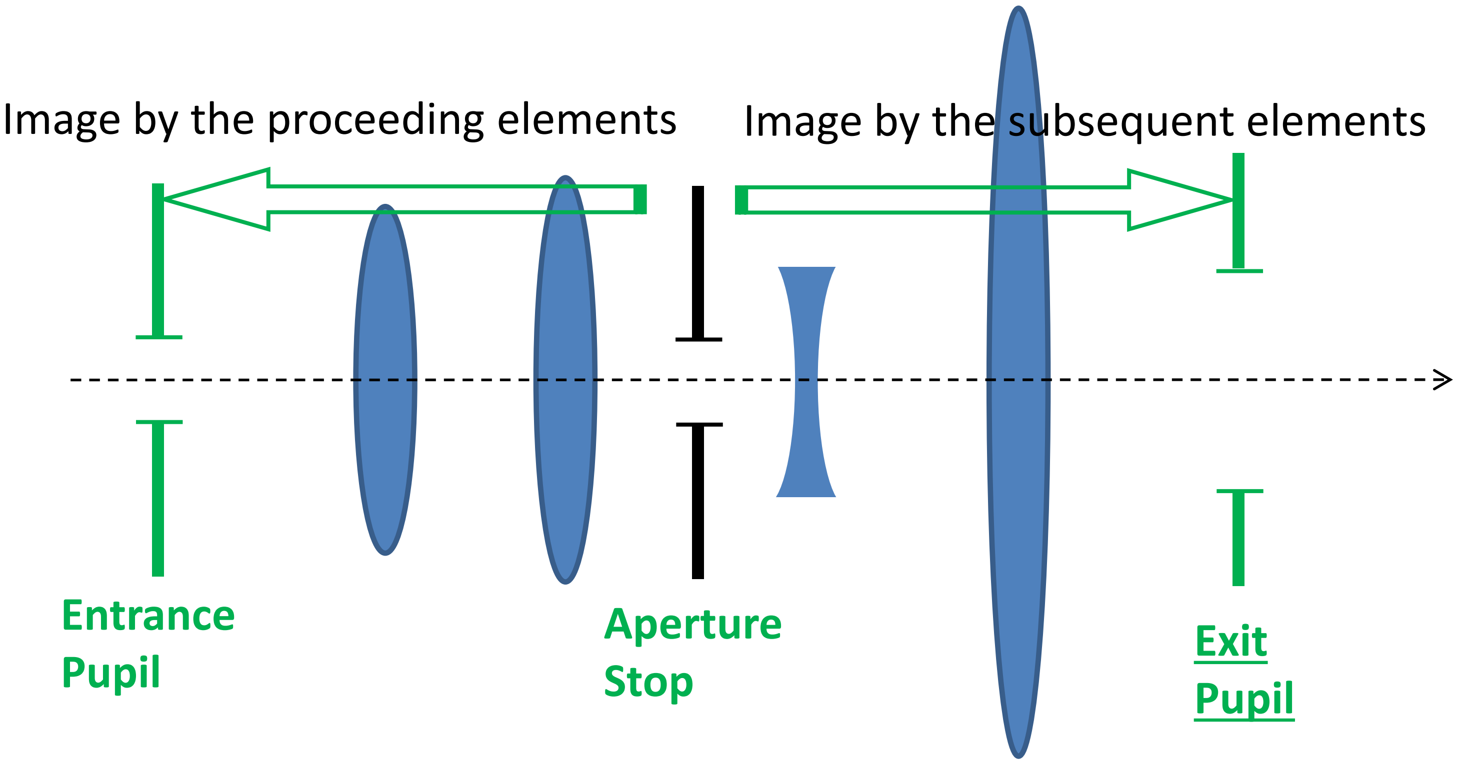


Exit Pupil (E_xP)

Once we find the aperture stop, we can also determine exit pupil (E_xP):

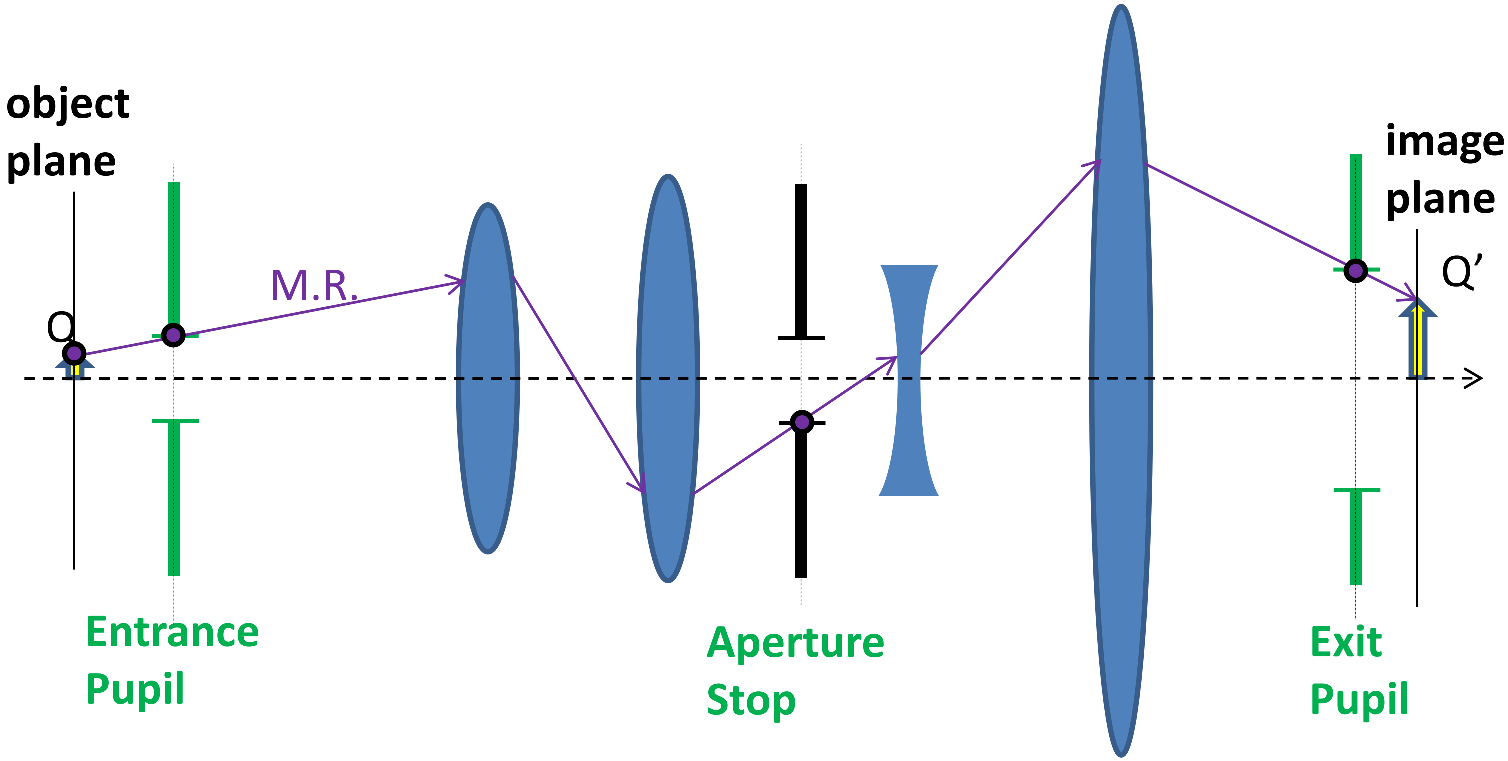
Exit pupil is defined as the image of the aperture stop by all the lenses after it

(i.e. to the right of AS – as if light travels from left to right)



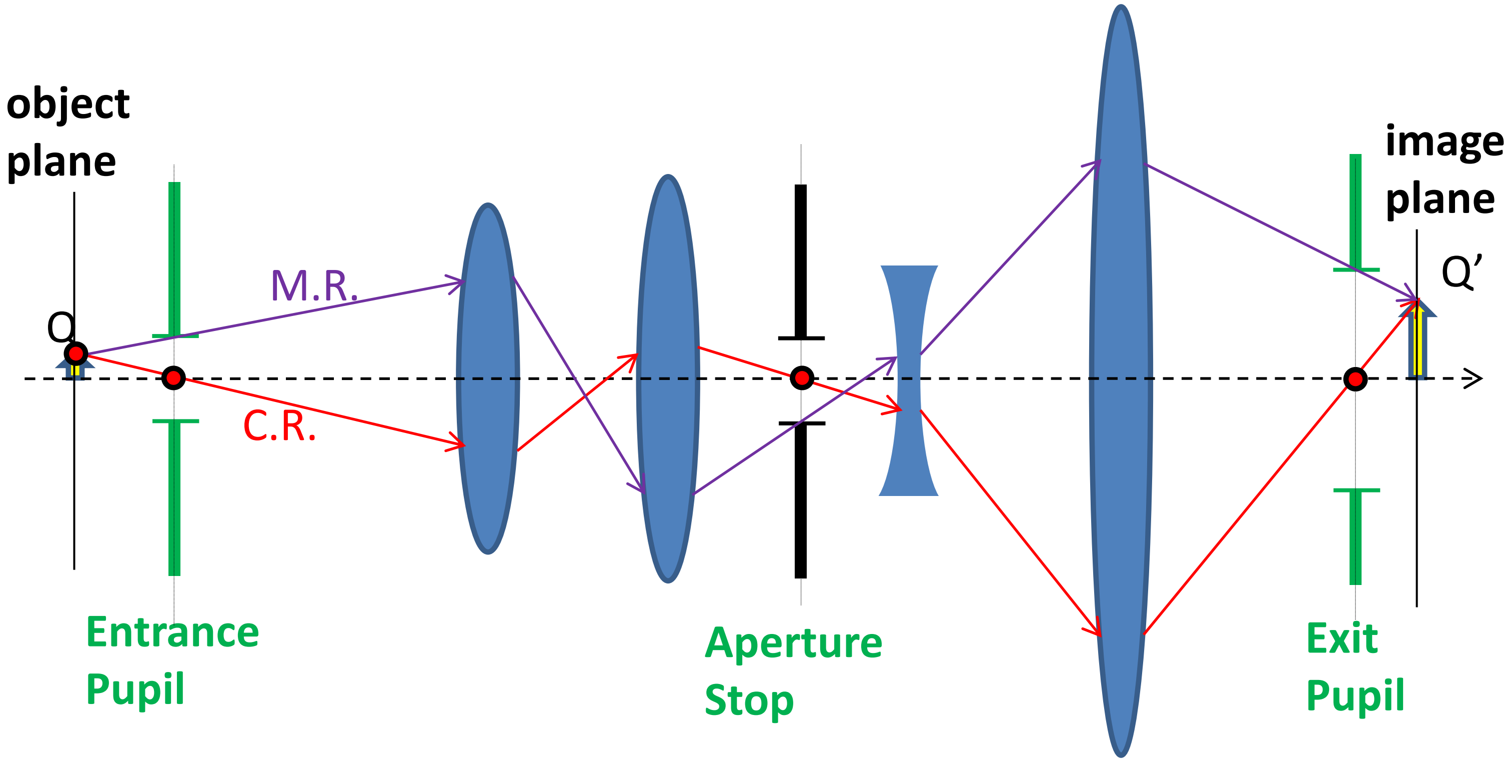
Note that Aperture Stop, E_nP and E_xP are on conjugate planes!!

Marginal Rays



- Lets chose a ray starting from an off-axis point, such as at Q in this example
- The light ray that passes through the **EDGE of the aperture stop (AS)** is called **marginal ray**
- The marginal ray will ALSO pass through the **EDGE** of the **exit pupil (E_xP)** and **entrance pupil (E_nP)** because these three planes are conjugated with each other.

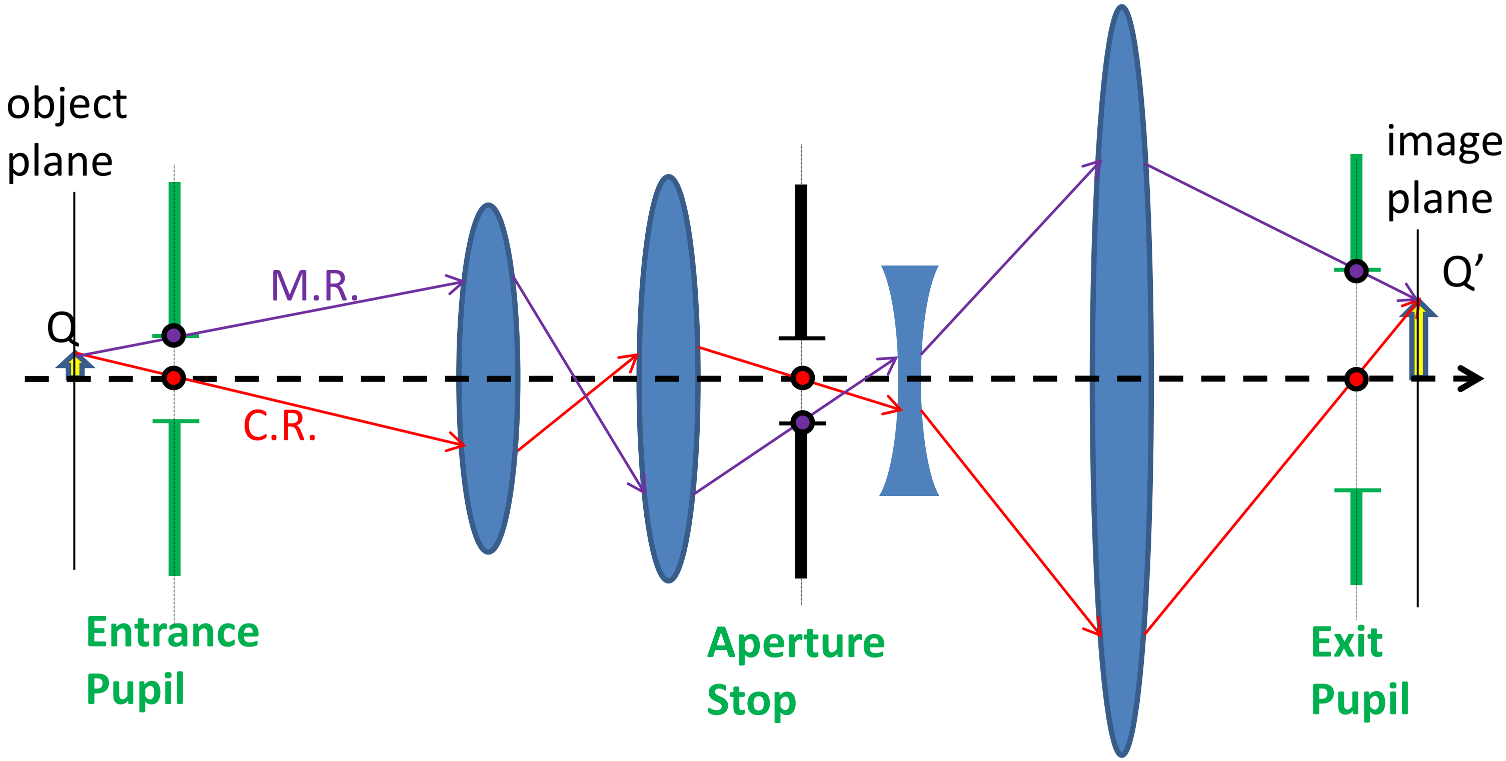
Chief Rays



- Lets chose a ray starting from an off-axis point in the object plane, such as Q in this example
- The light ray which passes through the **CENTER of the aperture stop** (AS) is called **chief ray**
- After refraction, the chief ray will also pass through the **CENTER** of the **exit pupil (E_xP)** and **entrance pupil (E_nP)** pupil because these three planes are **conjugated with each other**.

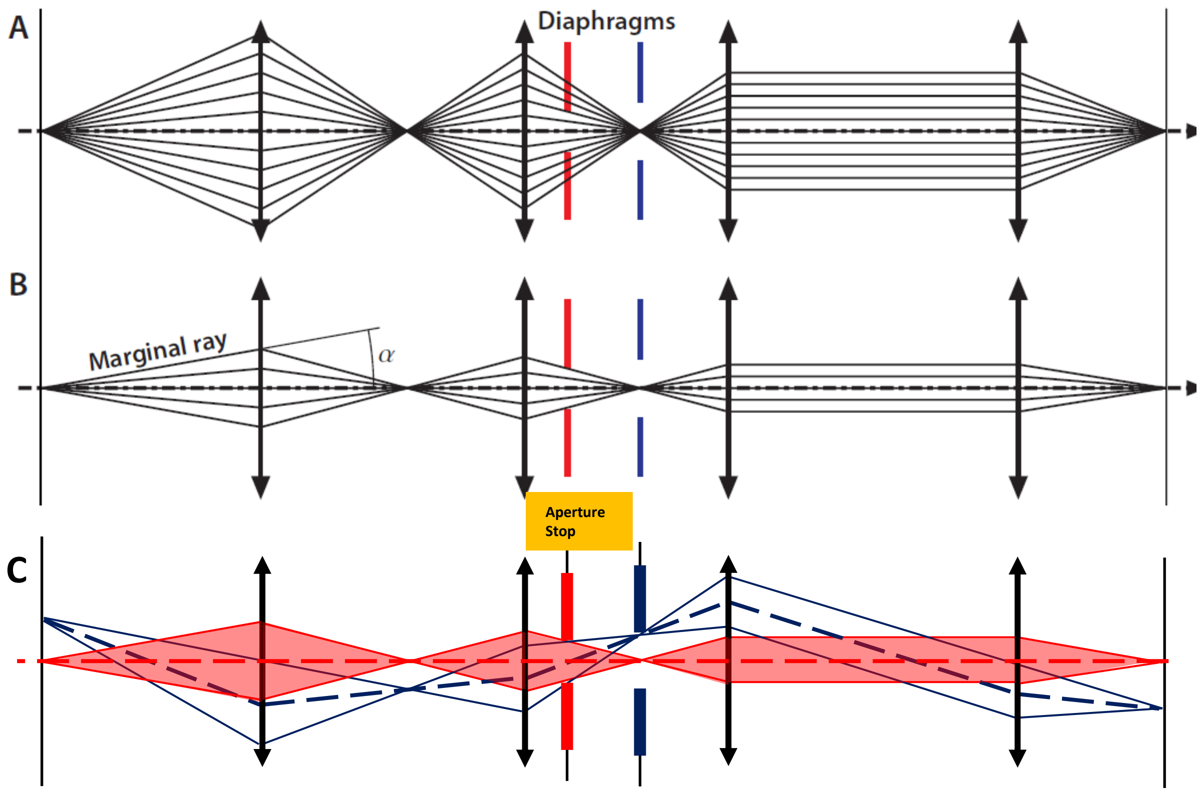
➔ CR & MR meet again at the image point (Q’), because object & image planes are also conjugated

Pupils and Marginal Ray & Chief Ray



- The pupils are located at the intersection of the chief rays with the optical axis
- The diameter of the pupils are given by the heights of the marginal rays at the pupil positions

Aperture Stops & Pupils and Marginal/Chief Rays



C:

Red-shaded = Axial ray bundle originating from the optical axis

Blue-region = Ray bundle originating from an extended point

Chief rays are indicated by **dashed lines** → they cross from the center of the **aperture stop**

Marginal rays are indicated by **solid lines** → they touch to the rims of the **aperture stop**

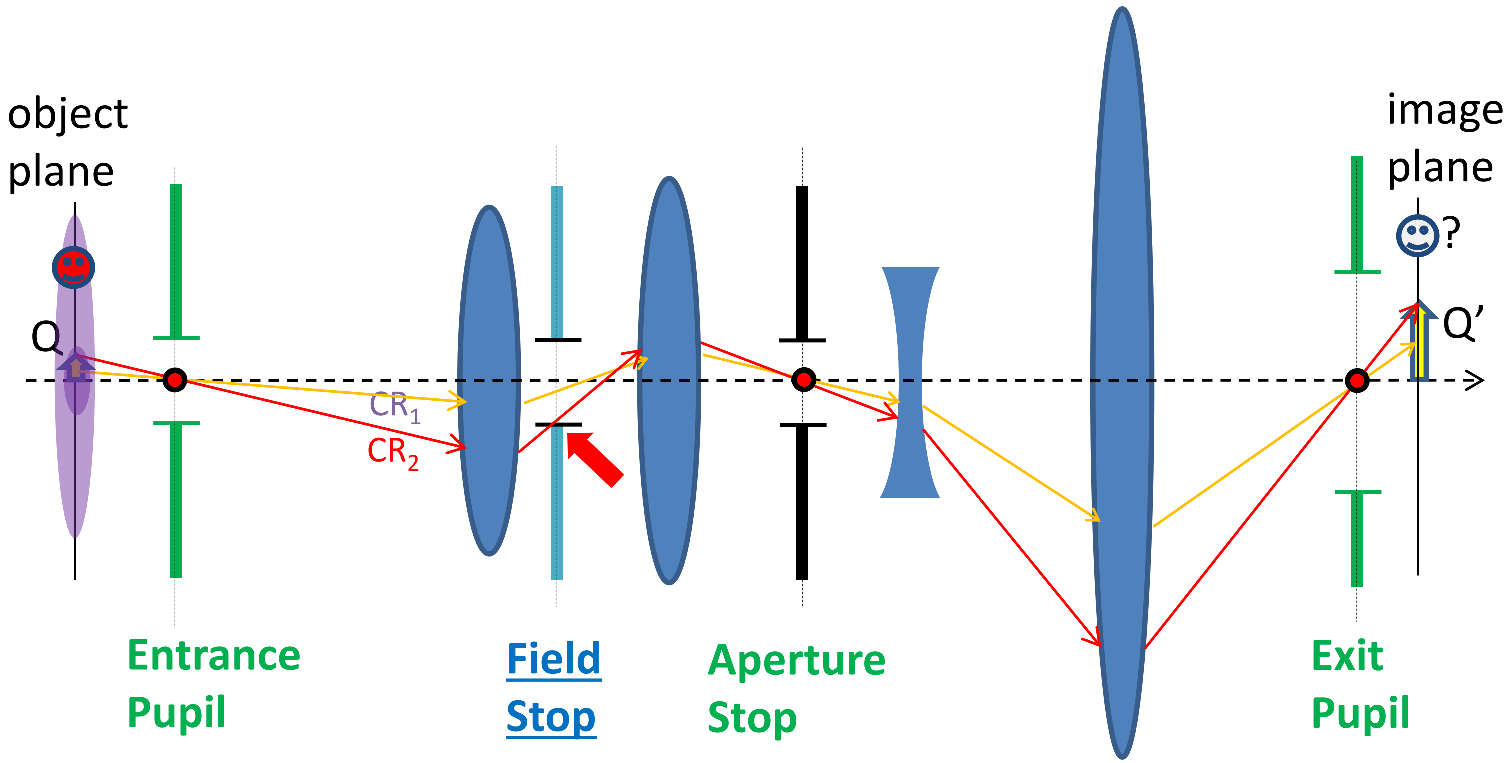
Field Stop

- An important question in microscopy: what is t**he maximum viewable area in the object plane? This is called field of view (FOV)** ➔ determines the size of the sample that we can "image"
- Let's place an object in the object plane (and its corresponding image in the image plane)

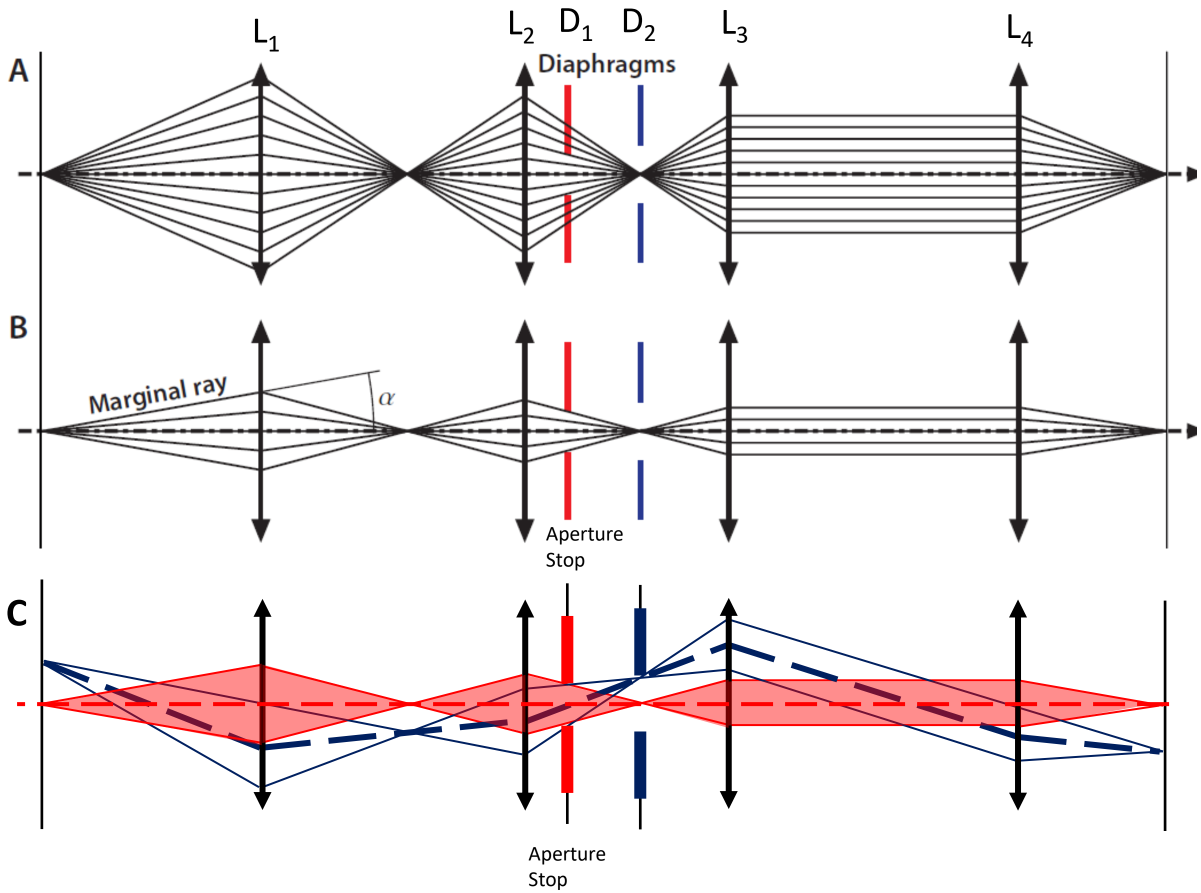
→ Consider possible **chief rays** starting from different object points and pointing towards the center of entrance pupil.

→ **CRs** passes through the **center** of the **aperture stop** and also **entrance** & **exit** pupils .

→ **Field stop** is the opening which **limits the chief rays**



General procedure: how to find the field stop?



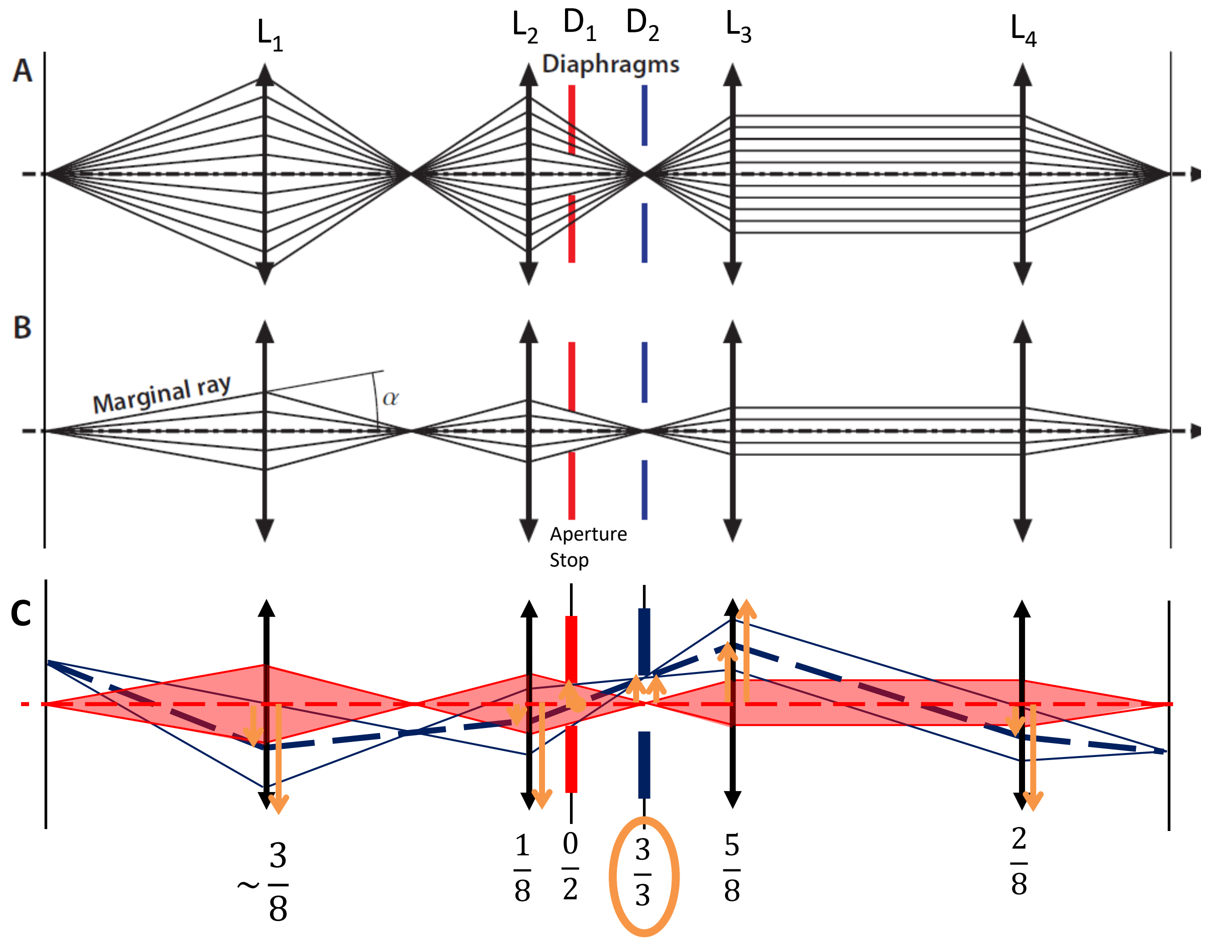
Remember:
MR passes through the
<u>EDGE of the aperture stop</u>

Remember:
CR passes through the
<u>CENTER of the aperture stop</u>

C :

- Trace **a chief ray** from an off-axis point **(for example blue dashed line in panel C)**
- Determine the ratio of the chief ray to the clear height of the each element

General procedure: how to find the field stop?



Remember:
MR passes through the <u>EDGE of the aperture stop</u>

Remember:
CR passes through the <u>CENTER of the aperture stop</u>

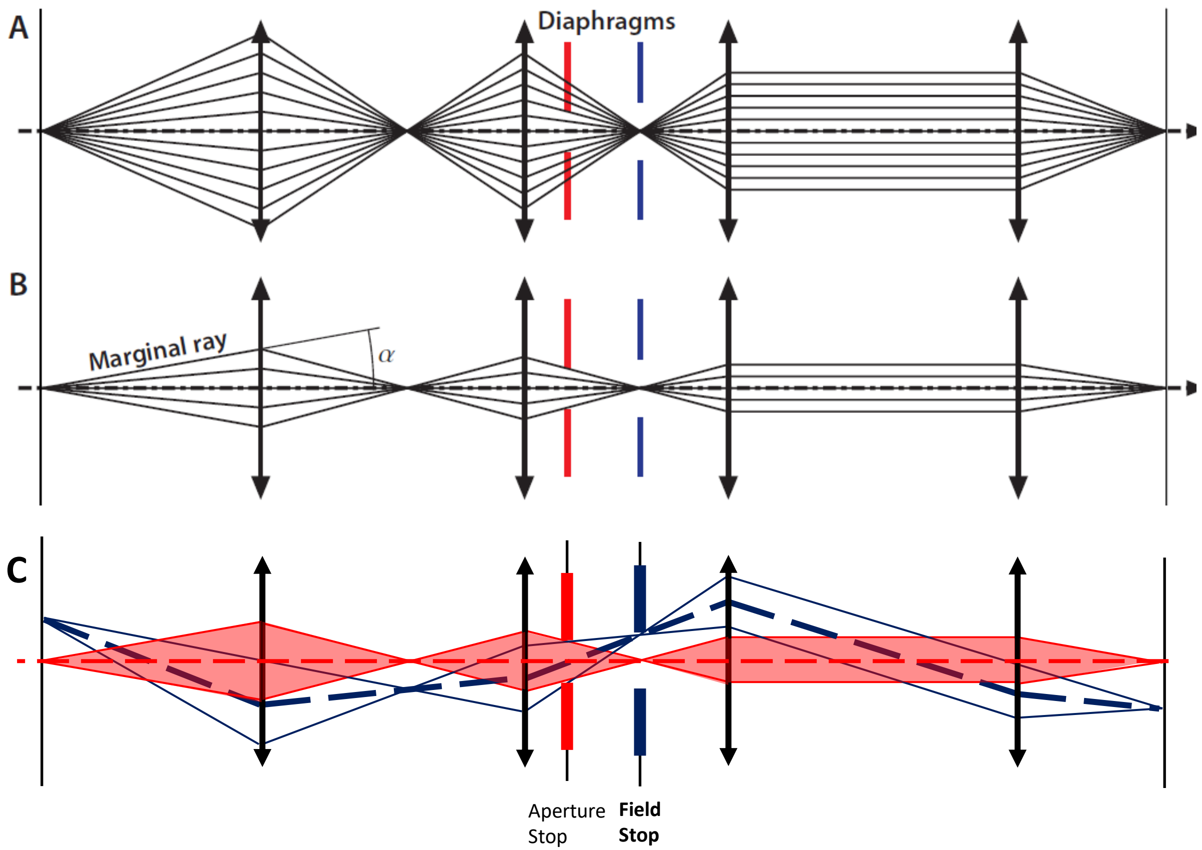
C :

- Trace **a chief ray** from an off-axis point **(i.e. blue dashed line in panel C)**
- Determine the ratio of the chief ray to the clear height of the each element

Diaphragm 2 (D_2) has the highest ratio: "1"

The numbers are approximated for illustration purposes

General procedure: how to find the field stop?



Remember:
MR passes through the <u>EDGE of the aperture stop</u>

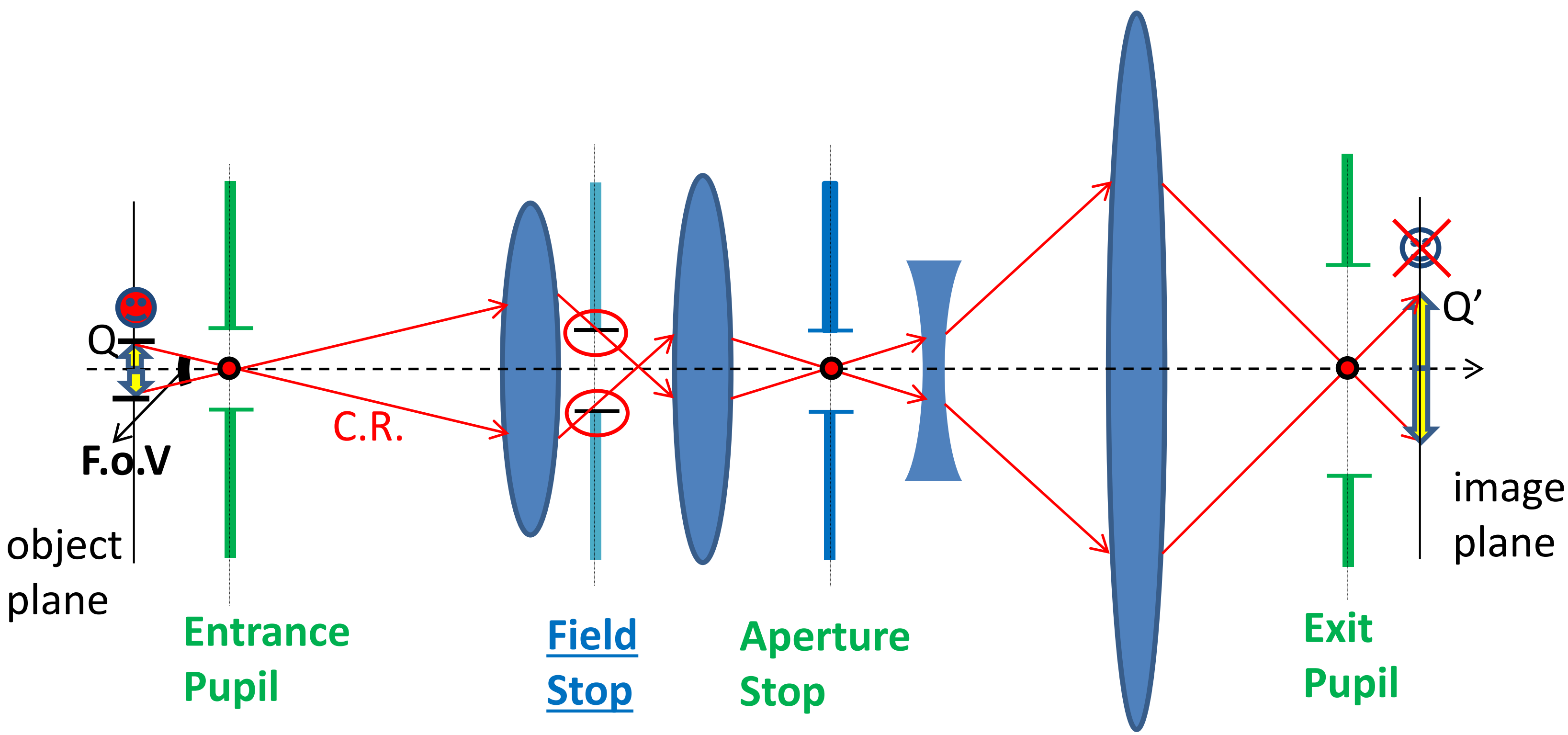
Remember:
CR passes through the <u>CENTER of the aperture stop</u>

C :

- Trace **a chief ray** from an off-axis point **(i.e. blue dashed line in panel C)**
- Determine the ratio of the chief ray to the clear height of the each element
- The largest ratio indicates the **most limiting element for chief rays** propagating through the system
- This element is called **field stop**

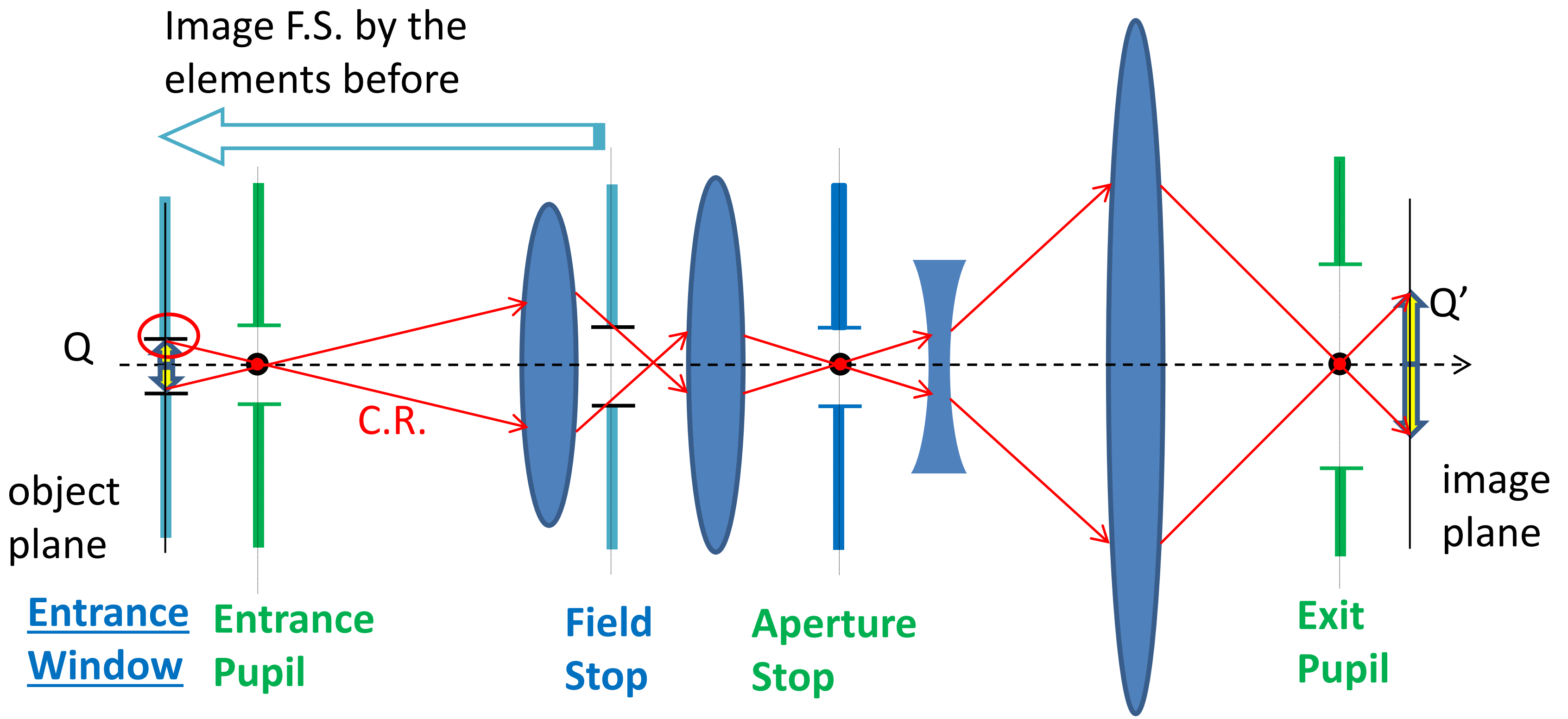
Field Stop defines the FIELD OF VIEW (F.o.V)

- Field stop (FS) is the opening which limits the chief rays.
- Field stop determines the boundaries of the "**visible**" objects.



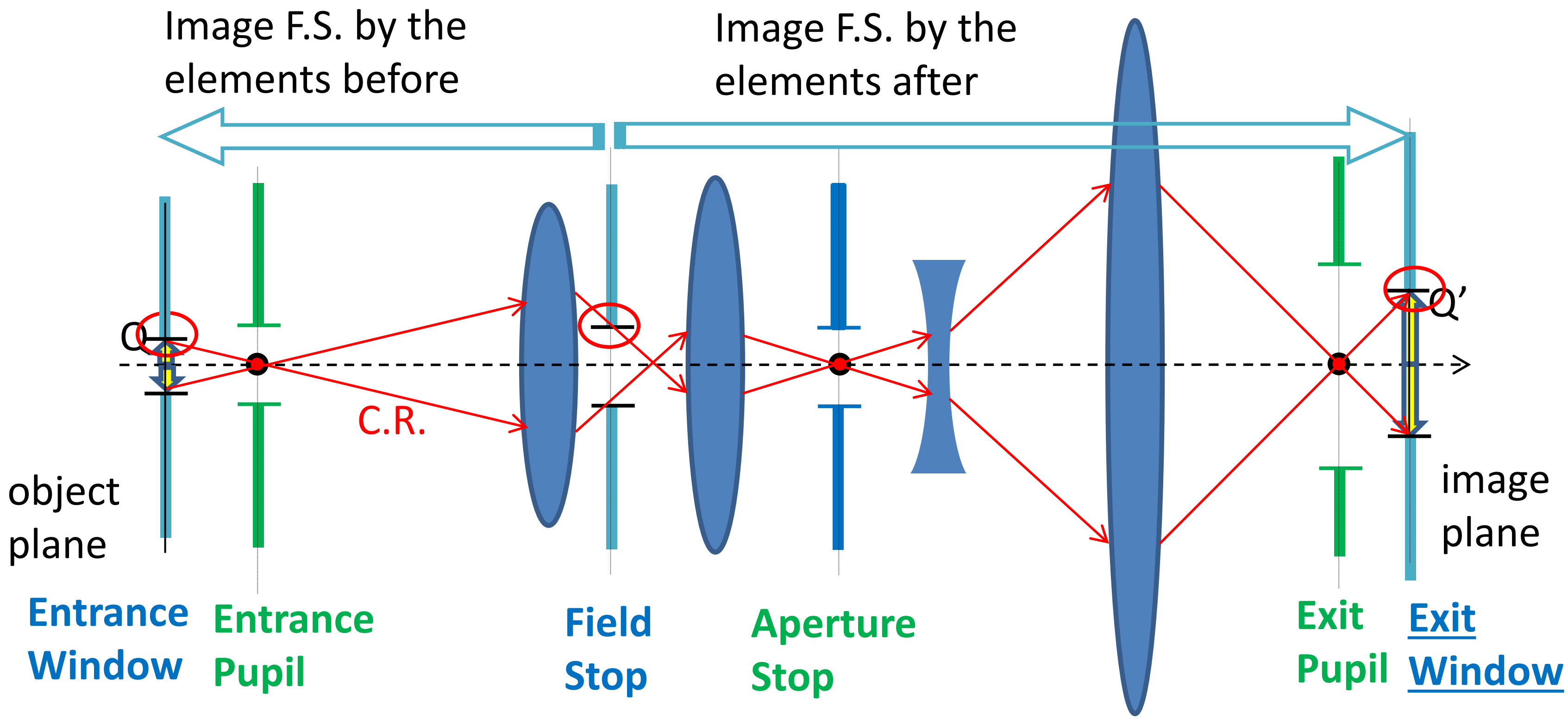
Entrance Window (E_nW)

- **Once you find the field stop, then determine E_nW:**
- **Entrance window is defined to be the image of the field stop by all the lenses before F.S.** **(i.e. to the left of F.S. - if light travels from left to right)**

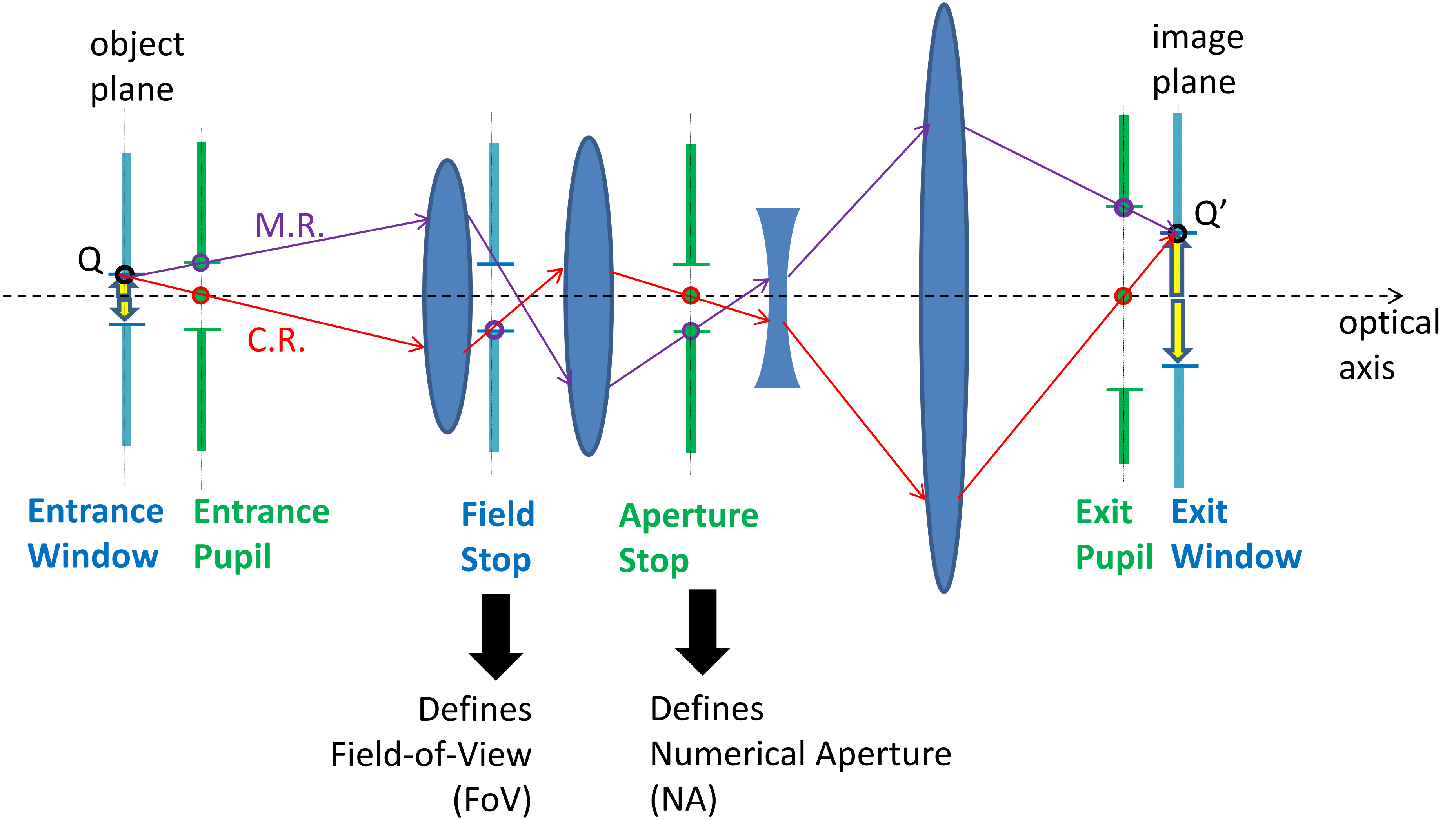


Exit Window (E_xW)

- **Once you find the field stop, then also determine E_xW:**
- **Exit window is defined to be the image of the field stop by all the lenses after it (i.e. to the right of F.S. - if light travels from left to right)**
- **Recall that Exit Window, Field Stop and Entrance Window are conjugated.**



All Together



Summary of definitions

- **Object-image relationships:**
  - **Image** is located at the intersection of all rays emitted from an object after propagation through the optical system.
  - **To find the image location,** trace minimum 2 rays and determine where they intersect.
  - Object plane and image plane are conjugated with each other

- **Limitations in microscopy**
  - **Aperture stop** limits the angular spread of the ray cone in an optical system
  - **Field stop** limits the visible size of the object/image

- **Other image-object relationships (conjugated planes):**

  Aperture stop ←→ Entrance Pupil & Exit Pupil

  Field stop ←→ Entrance Window & Exit Window

⚠ Aperture ≠ Diaphragm!

- **Diaphragm**: It is a physically limiting object to the rays.
- **Aperture stop**: It is the most limiting element to the rays.

  Aperture stop does not have to be the diaphragm. It can be a diaphgram, a lens, … ..