

# Confocal microscopy course

7 HEC!

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2014



# Results of the quiz for the lecture 2 (basics in confocal microscopy)

## 1. Comments about the lecture

awesome/good

53%

too fast at the end

27%

bad/confusing

20%

## 2. would you like more/less information in the lecture?

less

the same

more

no

6%

60%

12%

yes

less for 

## 3. Average level of boredom (scale 0-10)

0.6!

## 4. Will the information from the lecture be useful for you

yes

93%

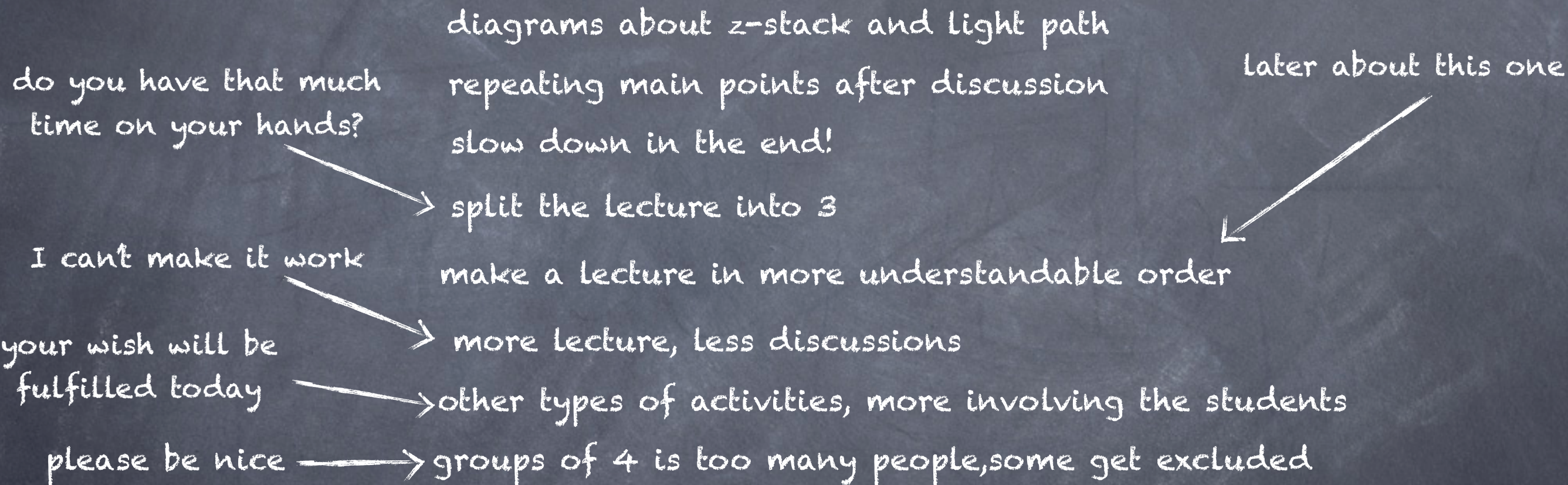
maybe/some of it

7%



# Results of the quiz for the lecture 2 (basics in confocal microscopy)

## 4. what would you teach differently



## 5. would you like to be your student

yes  
83%

no!  
17%

not the most  
important feature

“prefer to have a highly informed student”

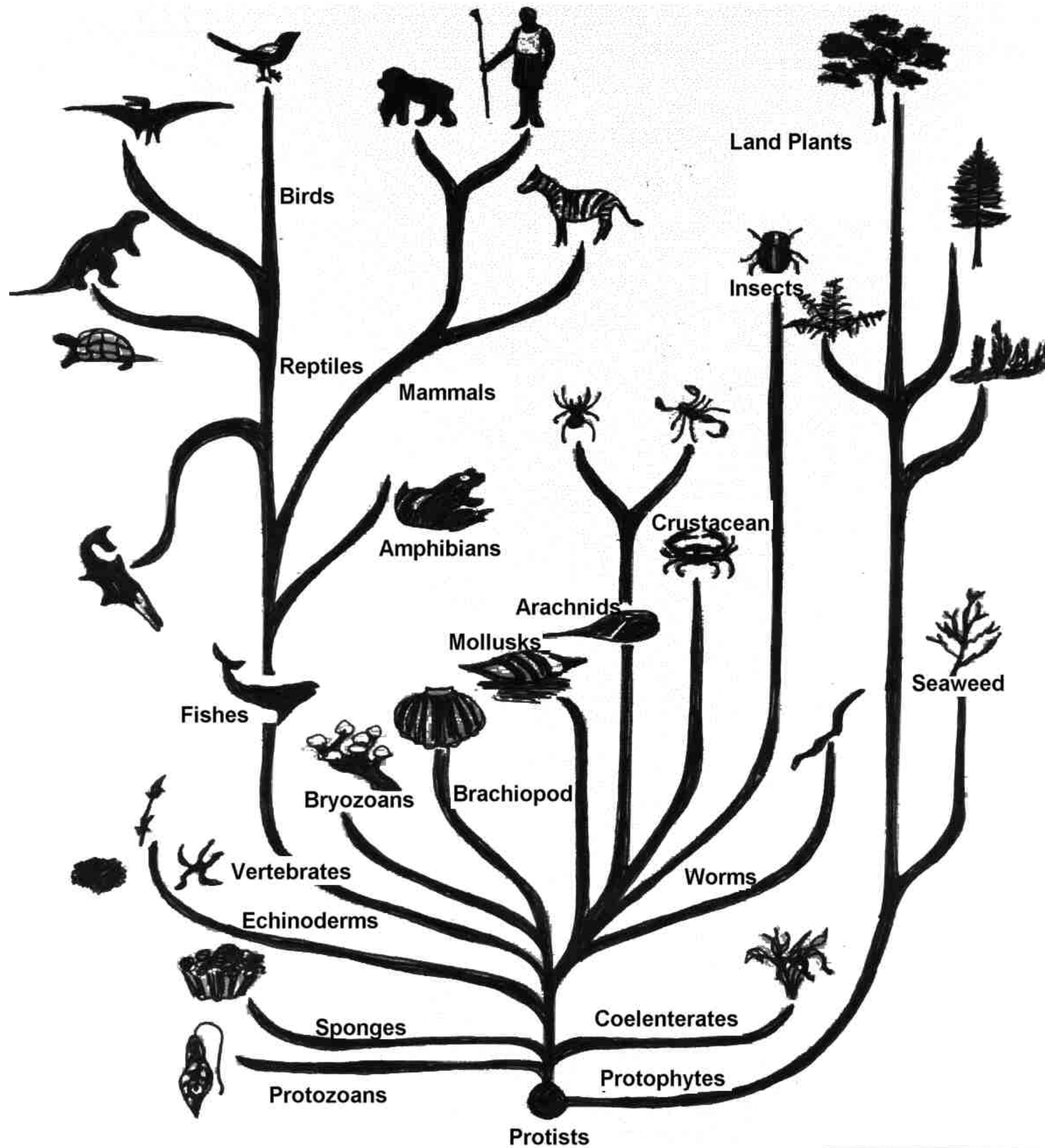


what was the logic behind the structure of the last lecture

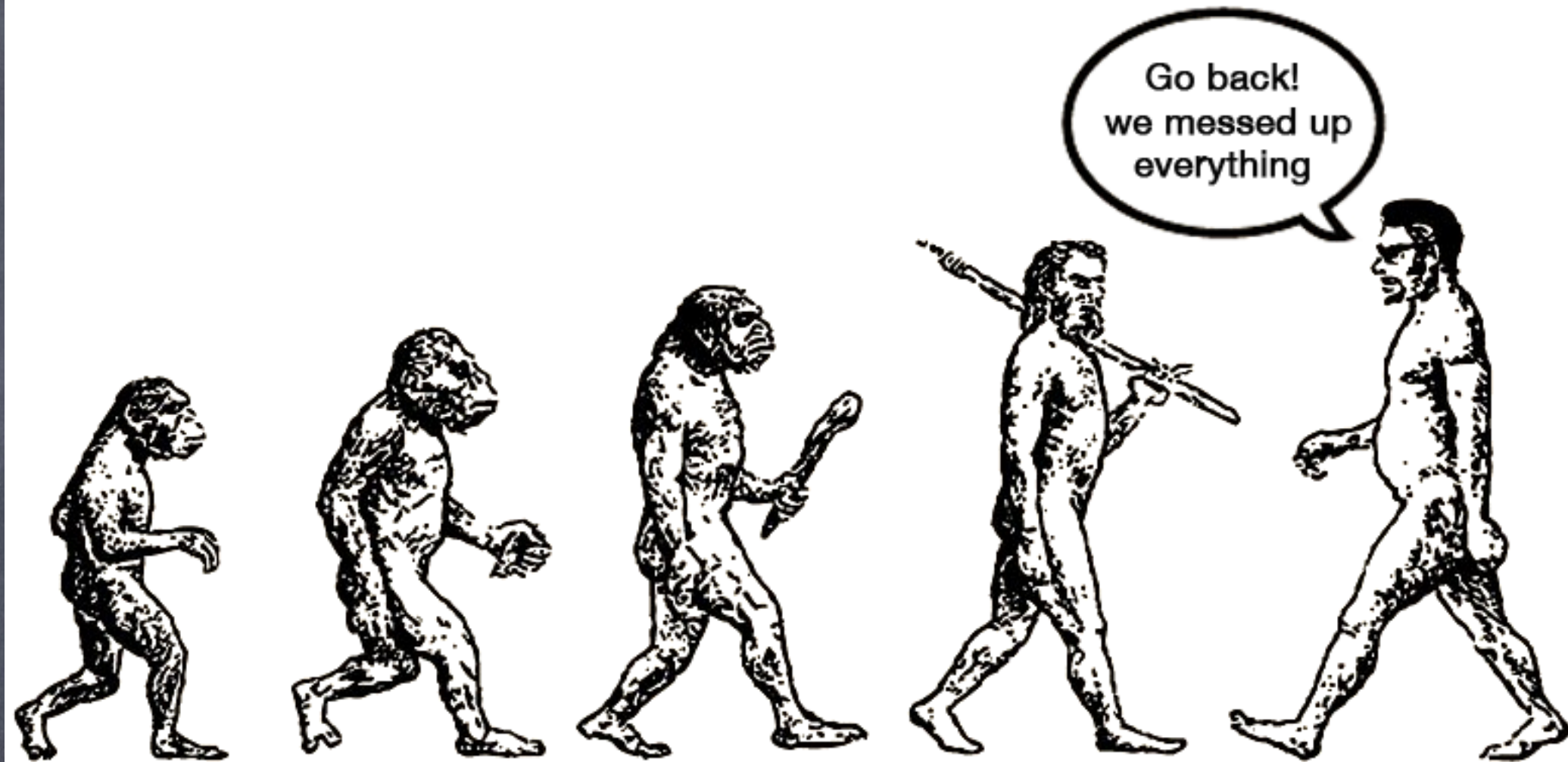
confocal didn't pop into existence from nothing

it "evolved"



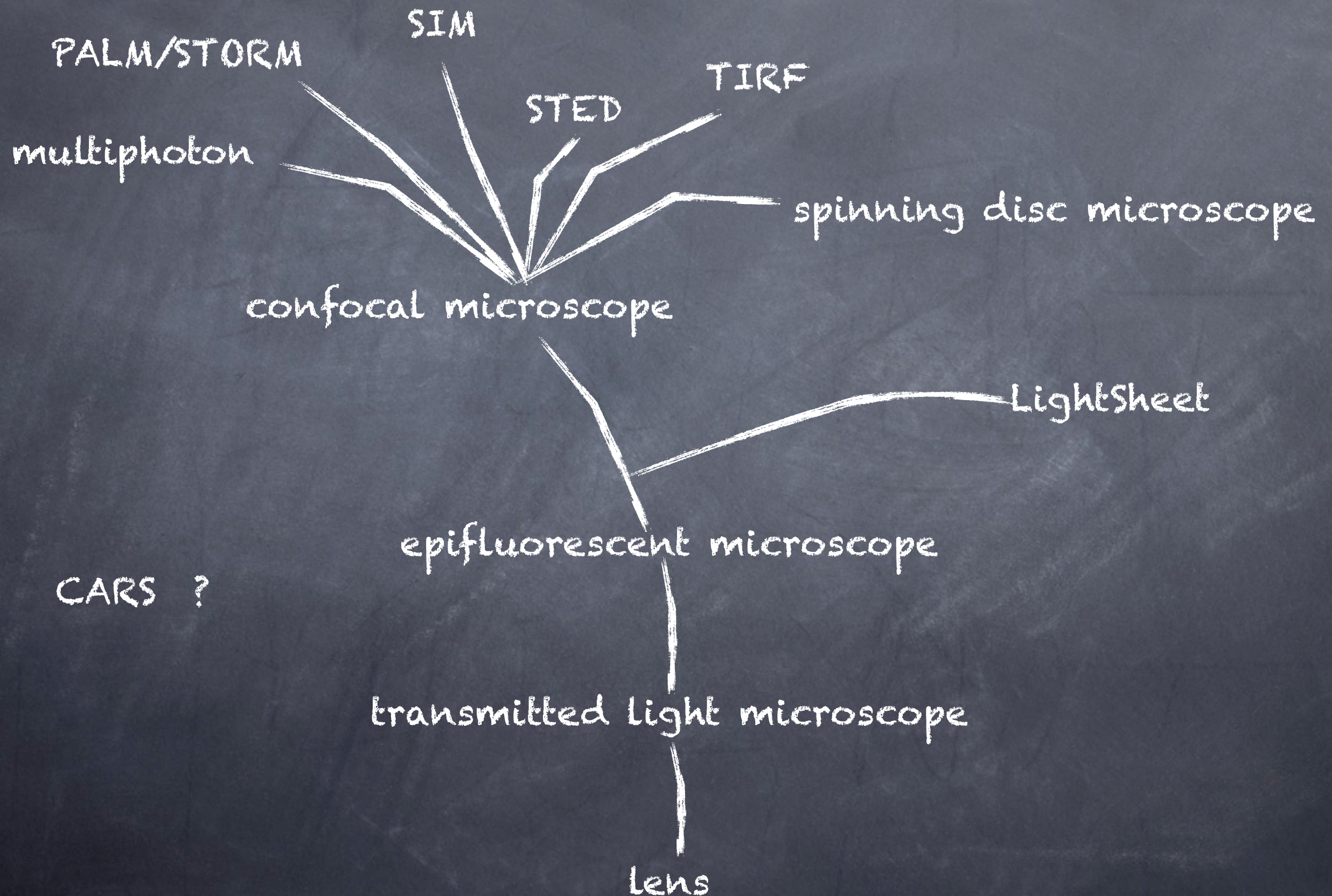




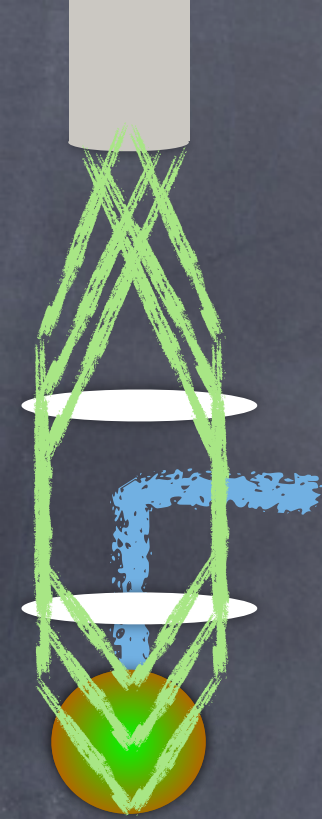


confocal evolved from an epifluorescent microscope



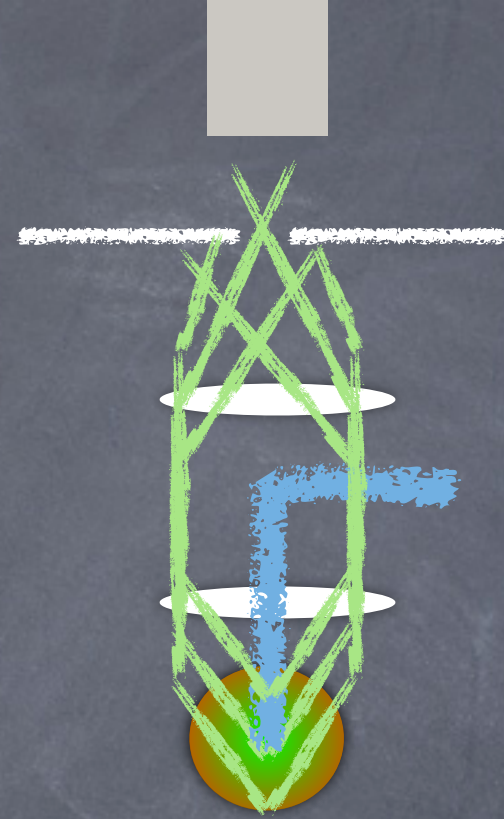






CM = confocal microscope

regular microscope + a pinhole



CLSM = confocal laser scanning microscope

regular microscope + a pinhole + lasers + PMT



visualising very small  
portion of a sample



CLSM

confocal laser scanning microscope

regular microscope      lasers      PMT      pinhole



you will need to find how to control it from the software



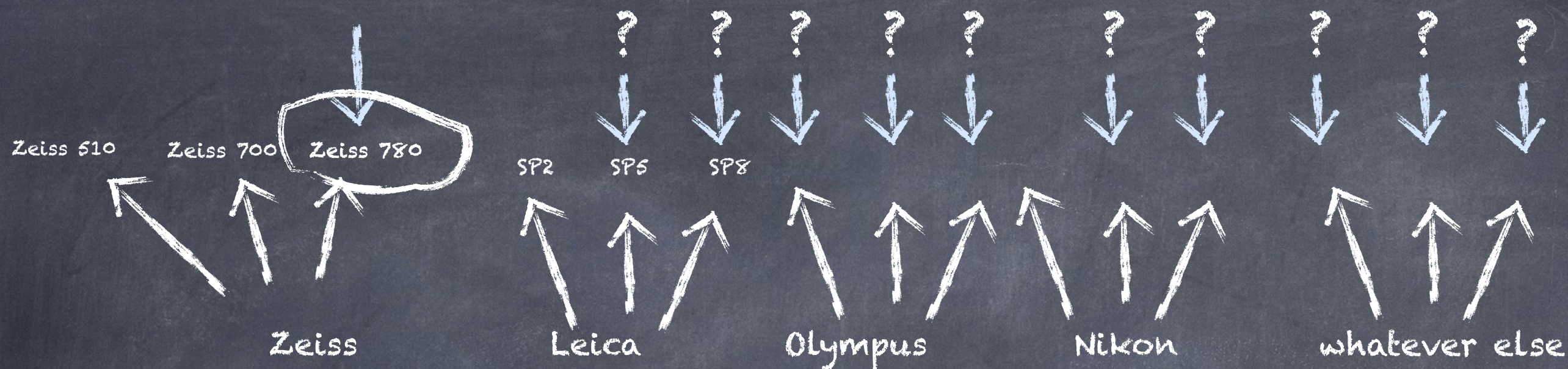
Zeiss 780

mounted on an **inverted** epifluorescent microscope

Axiovert







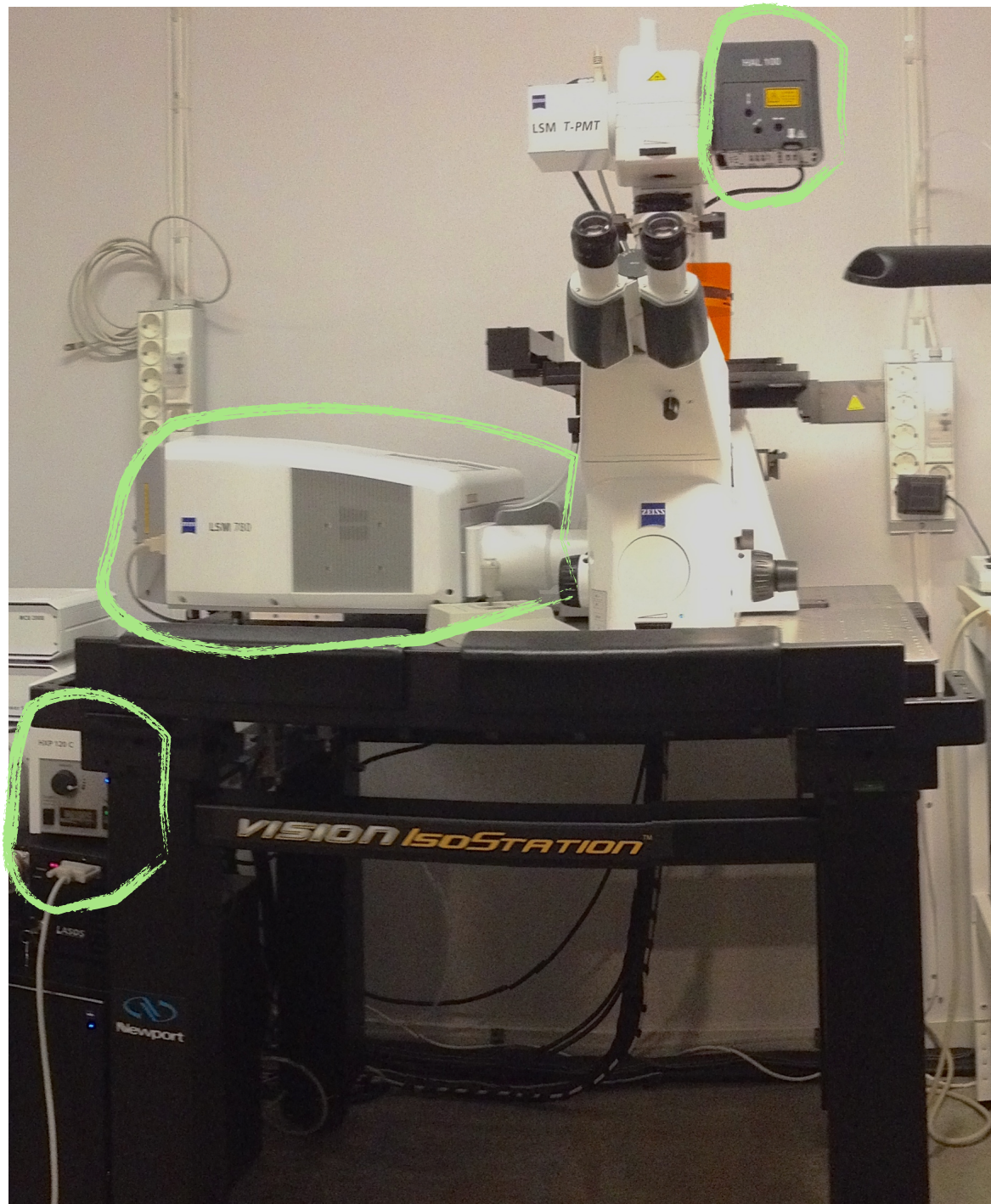
basics of confocal microscopy

basics of light microscopy

Axiomes



what if the Zeiss 780 was  
mounted on an **upright** epifluorescent microscope



where will be the HAL lamp?  
(transmitted light)

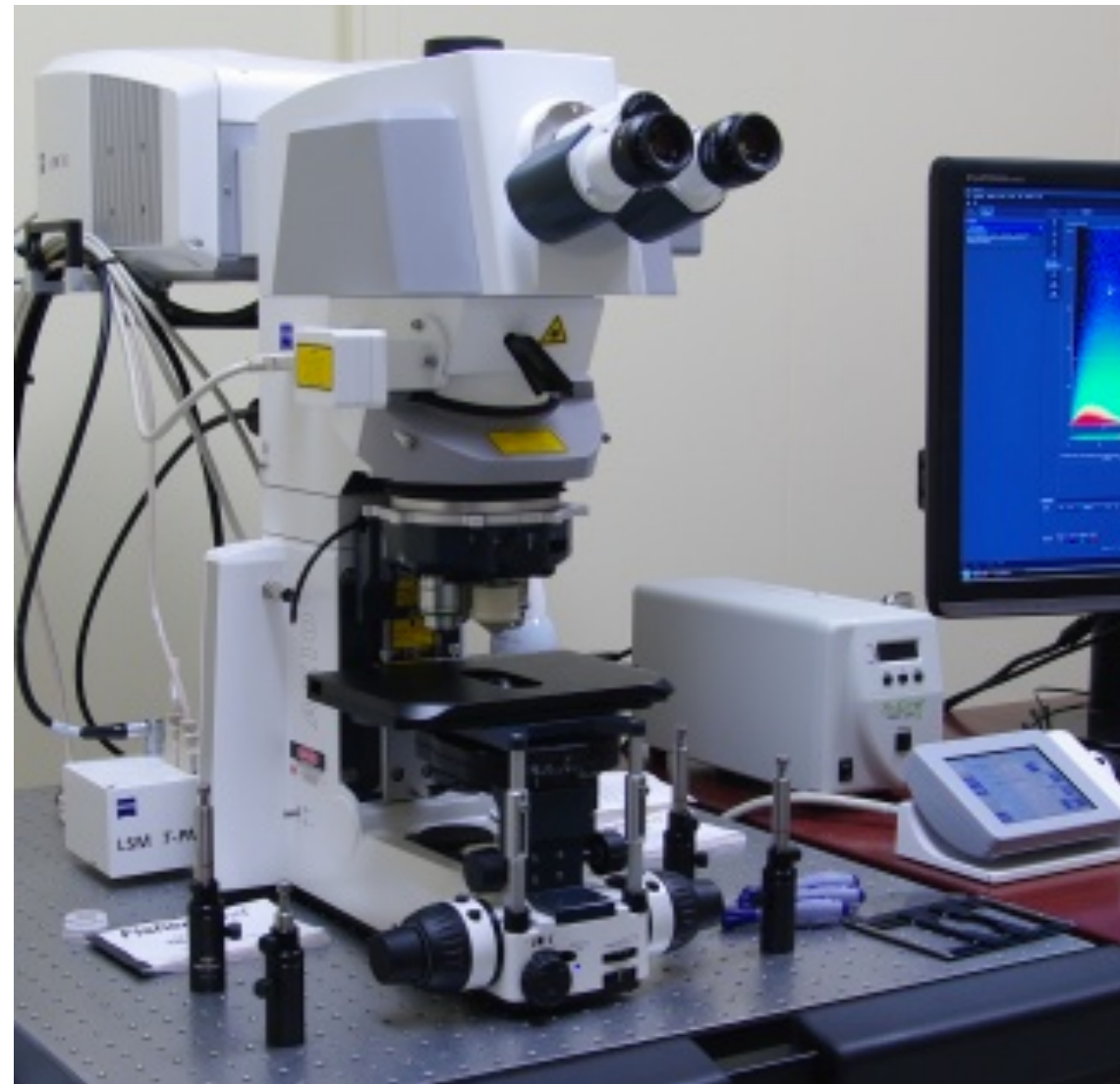
where will be the UV lamp?

where will be the confocal "box"?



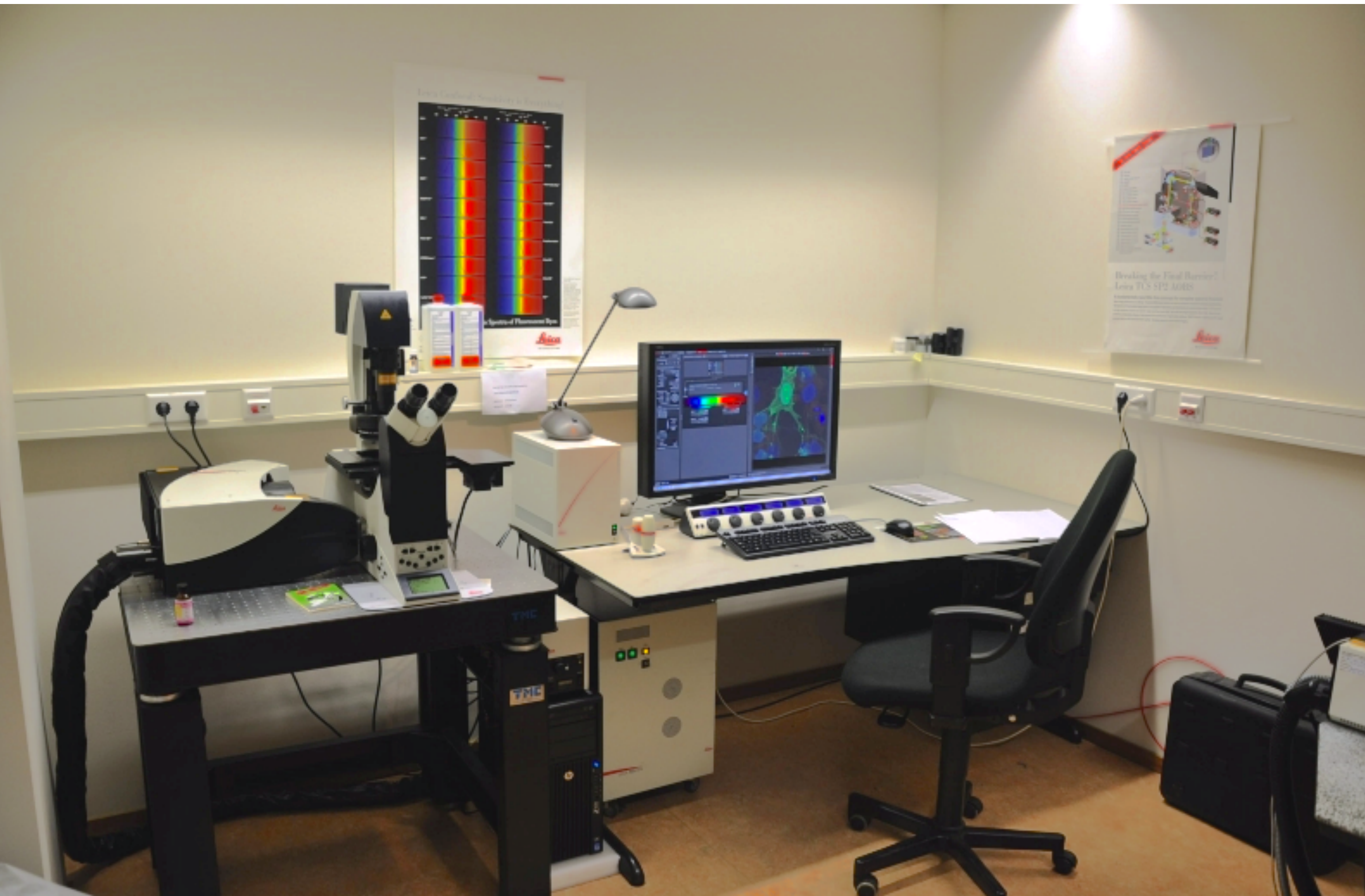
Zeiss 780

mounted on an **upright** epifluorescent microscope



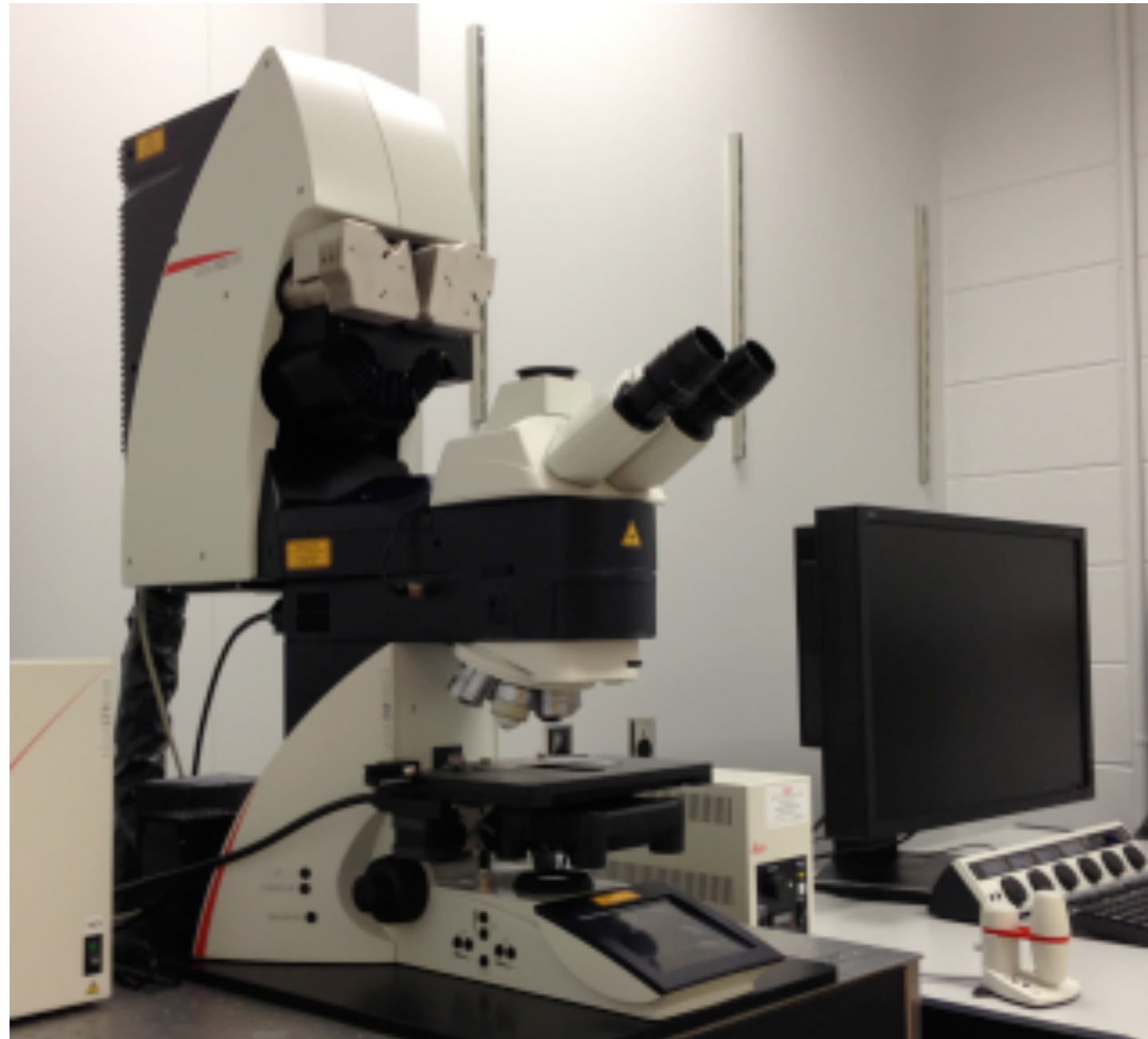


# Leica SP8 mounted on an inverted microscope





Leica SP8 mounted on an upright microscope





CLSM

Confocal Laser Scanning Microscopy

ok?

how do you know, that you know stuff?



please split in groups of two

draw an inverted epifluorescent microscope

mount a confocal part on it

I'm not kidding



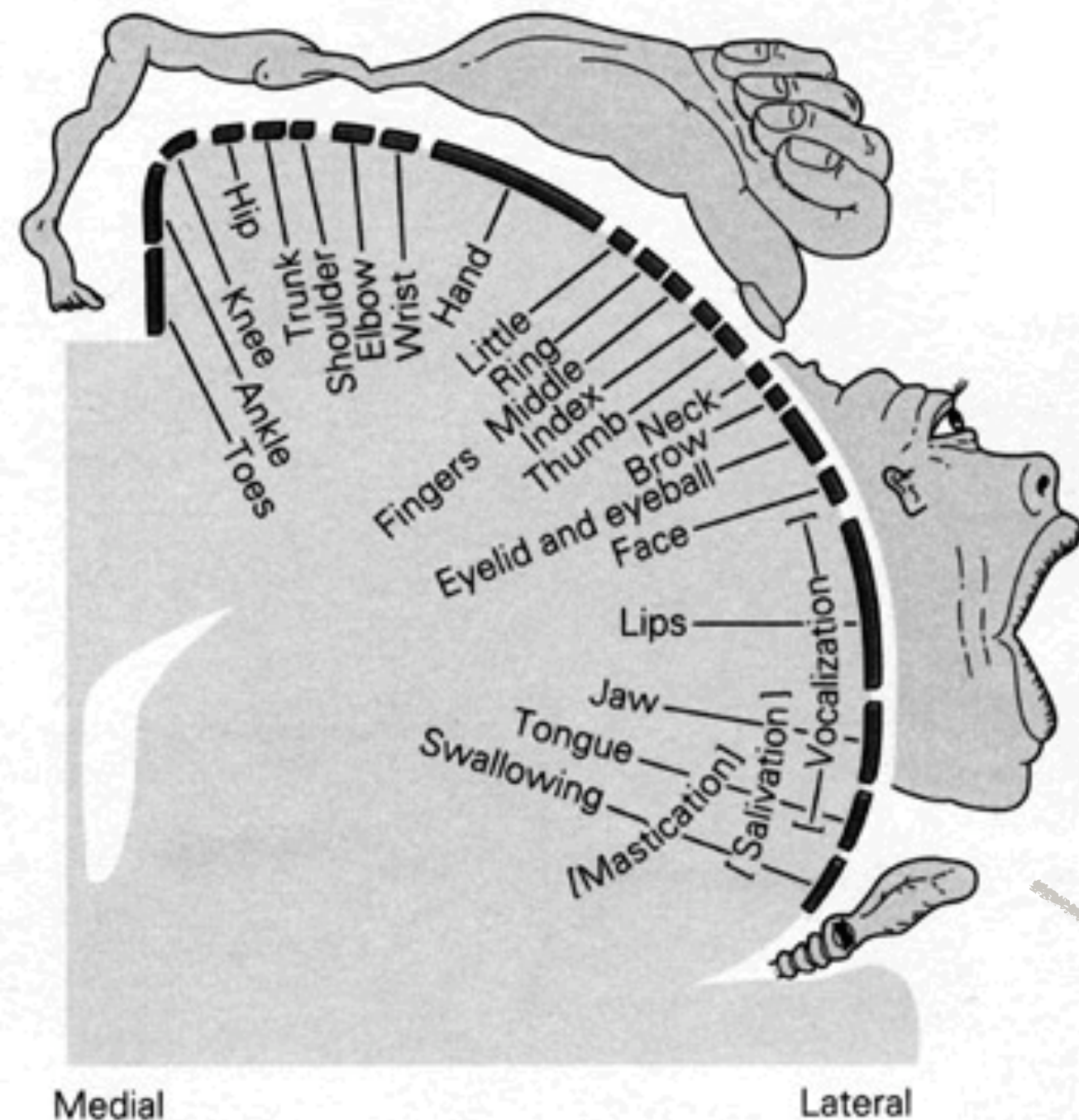
please mount a sample on your confocal

set up everything to detect GFP

can you see the signal?



this is how much attention  
a human brain devotes to  
the body parts



it looks like some  
information got lost



Please take a look at your scheme of a confocal

are any parts of it underrepresented?

please look up about these parts



score in amount of questions

Mohammad

Reza

Shirin

6:6

Jose

Catarina

Rita



please group with your people

Group 1

FRAP

Tania  
Andrea  
Daniel U  
Ylva  
Daniel J

Group 2

FRET

Maria  
Jose  
Shirin  
Reza  
Xue

Group 3

Optogenetics

Mohammad  
Masud  
Clement  
Rita  
Jing

Group 4

Photoconversion

Panisara  
Anna  
Catarina  
Leonor  
Abdul



# new competition in questioning reality

1. pick the topic of another group
2. please write down ALL the questions you can ask about this topic

you have about 5 minutes

Group 1

FRAP

Tania  
Andrea  
Daniel U  
Ylva  
Daniel J

Group 2

FRET

Maria  
Jose  
Shirin  
Reza  
Xue

Group 3

Optogenetics

Mohammad  
Masud  
Clement  
Rita  
Jing

Group 4

Photoconversion

Panisara  
Anna  
Catarina  
Leonor  
Abdul



your questions, please



now back to the topic assigned to your group

1. please discuss ALL the questions the other group asked about your topic

2. please write down ALL questions you can not answer

you have about 15 minutes

Group 1

FRAP

Tania  
Andrea  
Daniel U  
Ylva  
Daniel J

Group 2

FRET

Maria  
Jose  
Shirin  
Reza  
Xue

Group 3

Optogenetics

Mohammad  
Masud  
Clement  
Rita  
Jing

Group 4

Photoconversion

Panisara  
Anna  
Catarina  
Leonor  
Abdul



please group with your new people

Group 1

Tania  
Daniel J  
Jose  
Clement  
Anna

Group 2

Andrea  
Maria  
Rita  
Leonor  
Abdul

Group 3

Daniel U  
Xue  
Shirin  
Mohammad  
Panisara

Group 4

Ylva  
Reza  
Jing  
Masud  
Catarina



1. please tell your new group members about your topic
2. please discuss with your new group members any questions about your topic you couldn't answer
3. please discuss how each of the topics might be applied in the research of each of your group members

you have about 20 minutes

#### Group 1

Tania  
Daniel J  
Jose  
Clement  
Anna

#### Group 2

Andrea  
Maria  
Rita  
Leonor  
Abdul

#### Group 3

Daniel U  
Xue  
Shirin  
Mohammad  
Panisara

#### Group 4

Ylva  
Reza  
Jing  
Masud  
Catarina

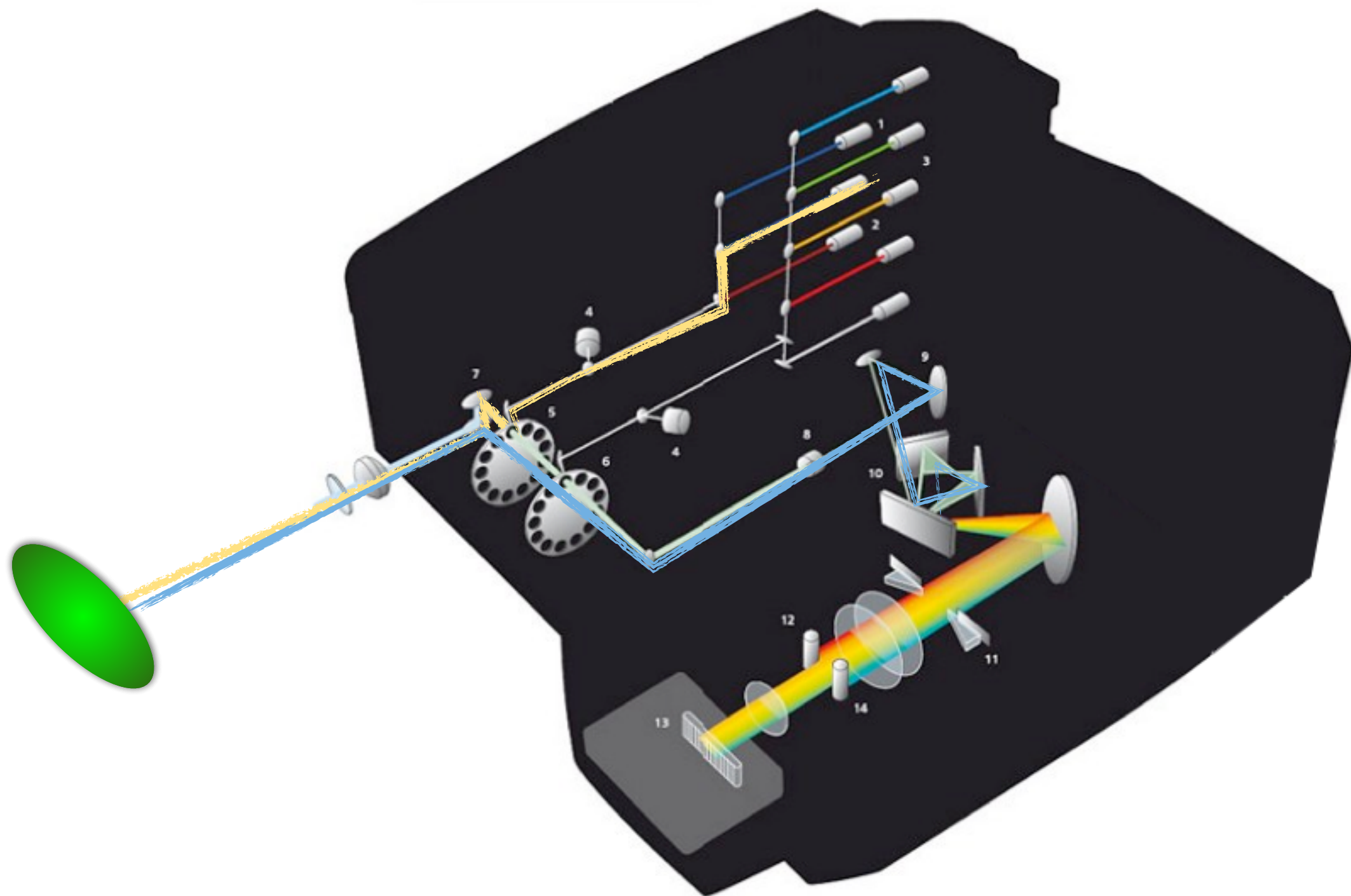


do you have any questions left  
unanswered?



from the previous lecture

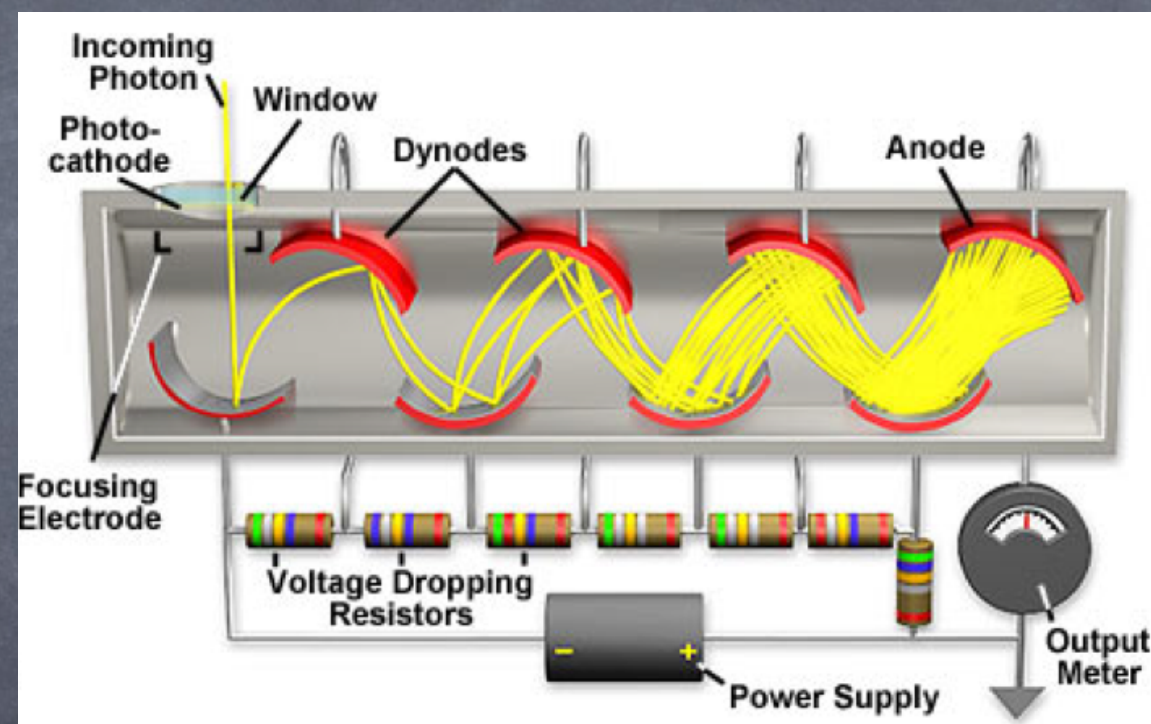




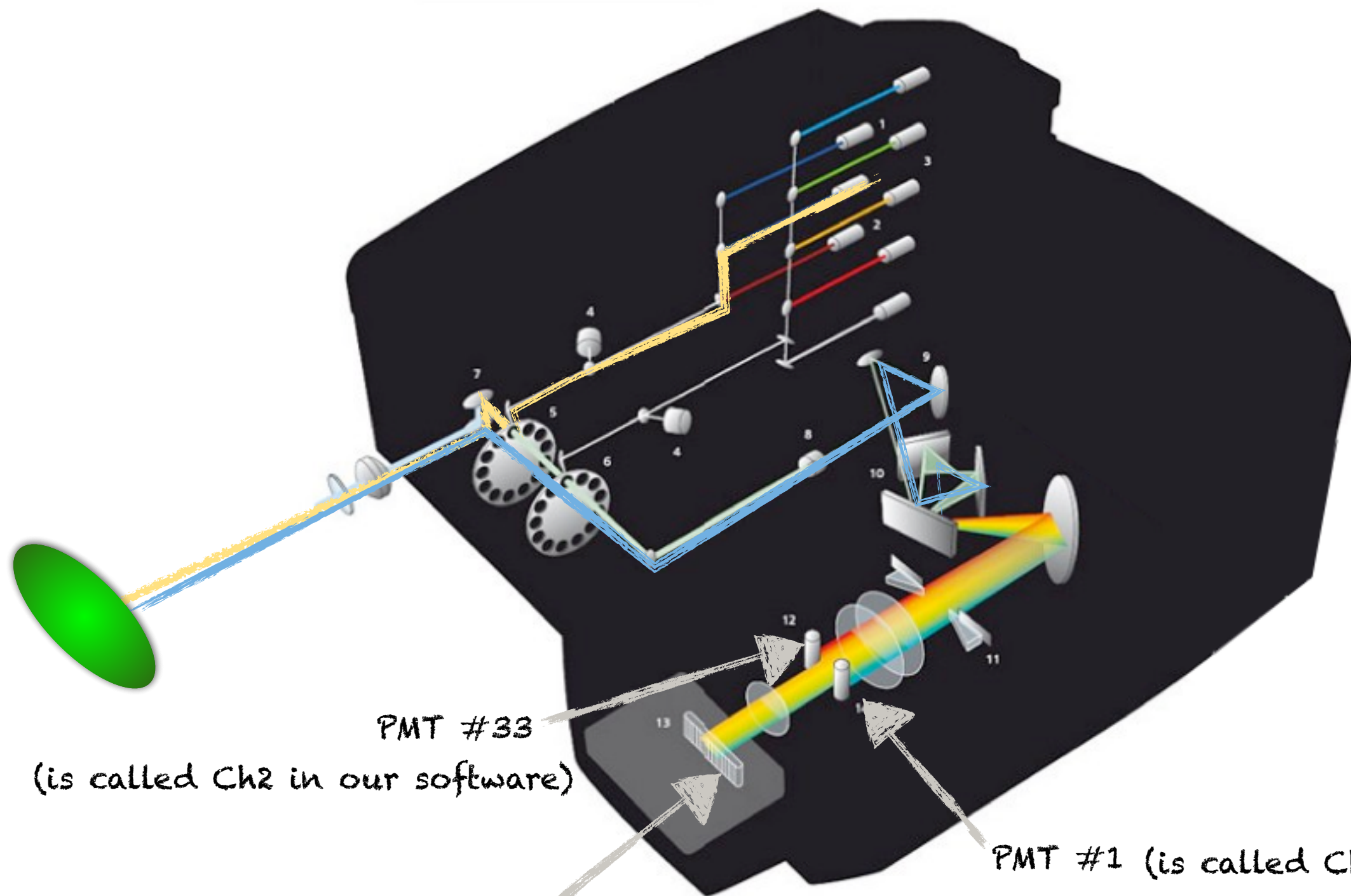


# Detector = PMT

## Photomultiplier tube







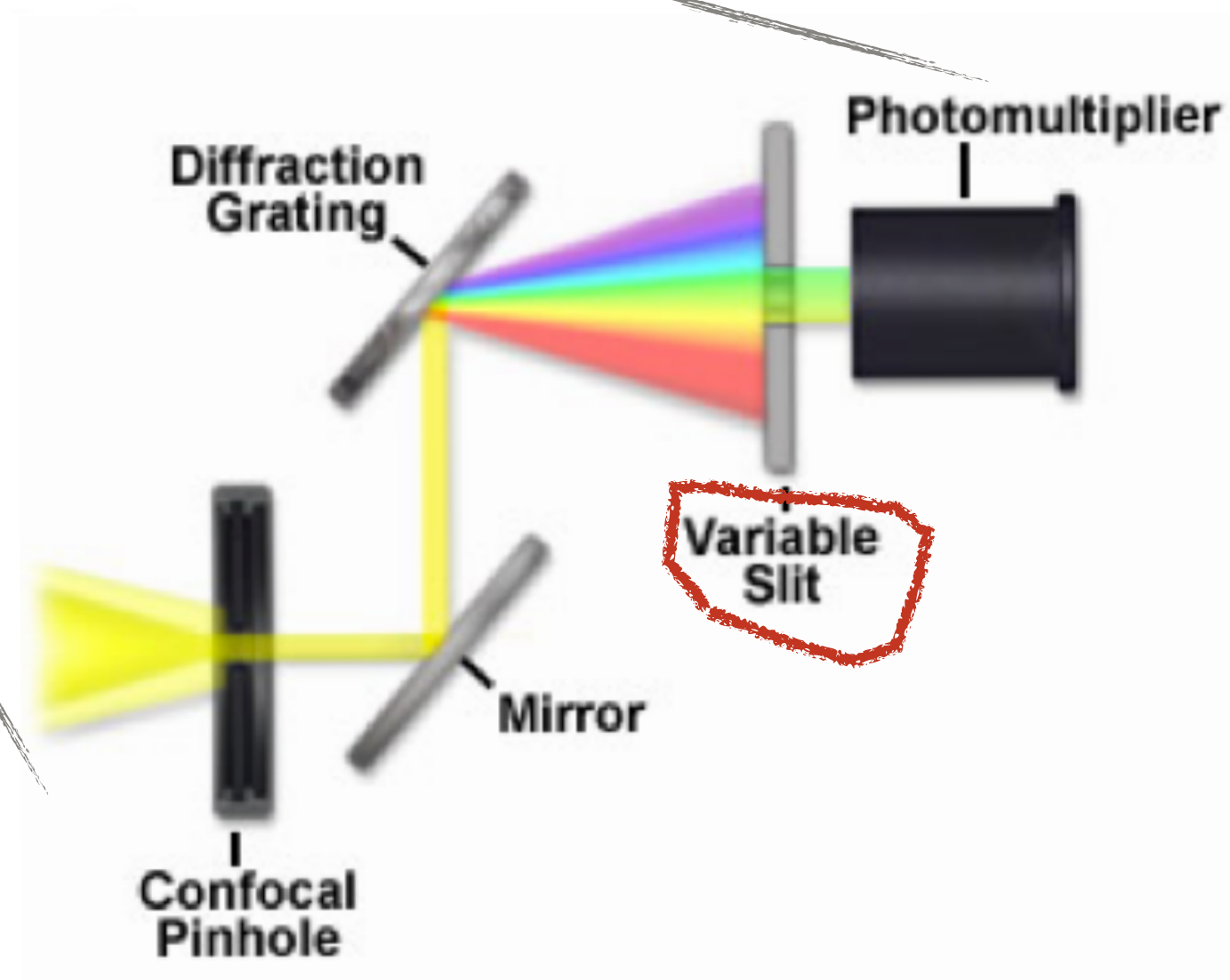
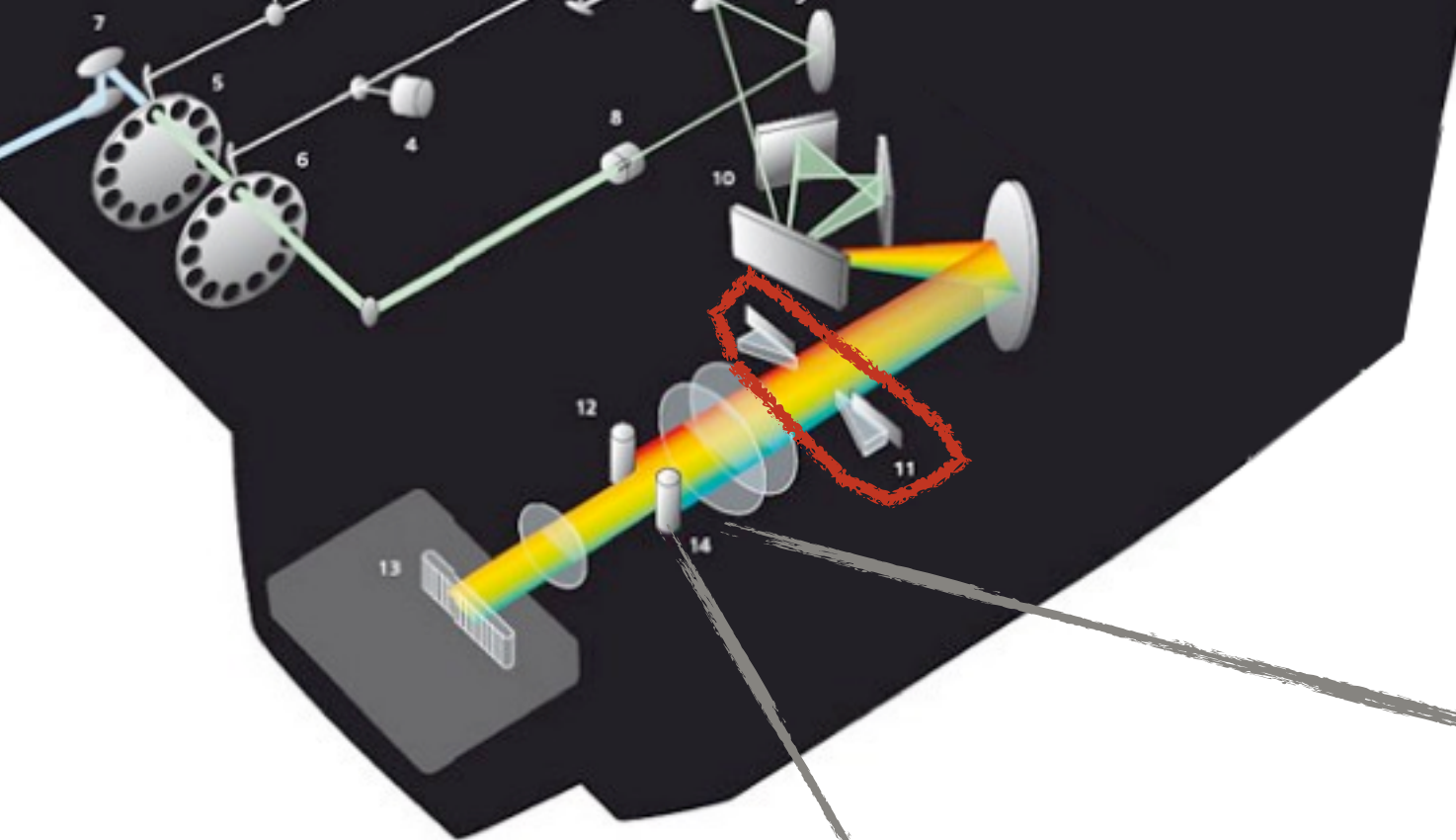
PMT #33  
(is called Ch2 in our software)

PMT #1 (is called Ch1 in our software)

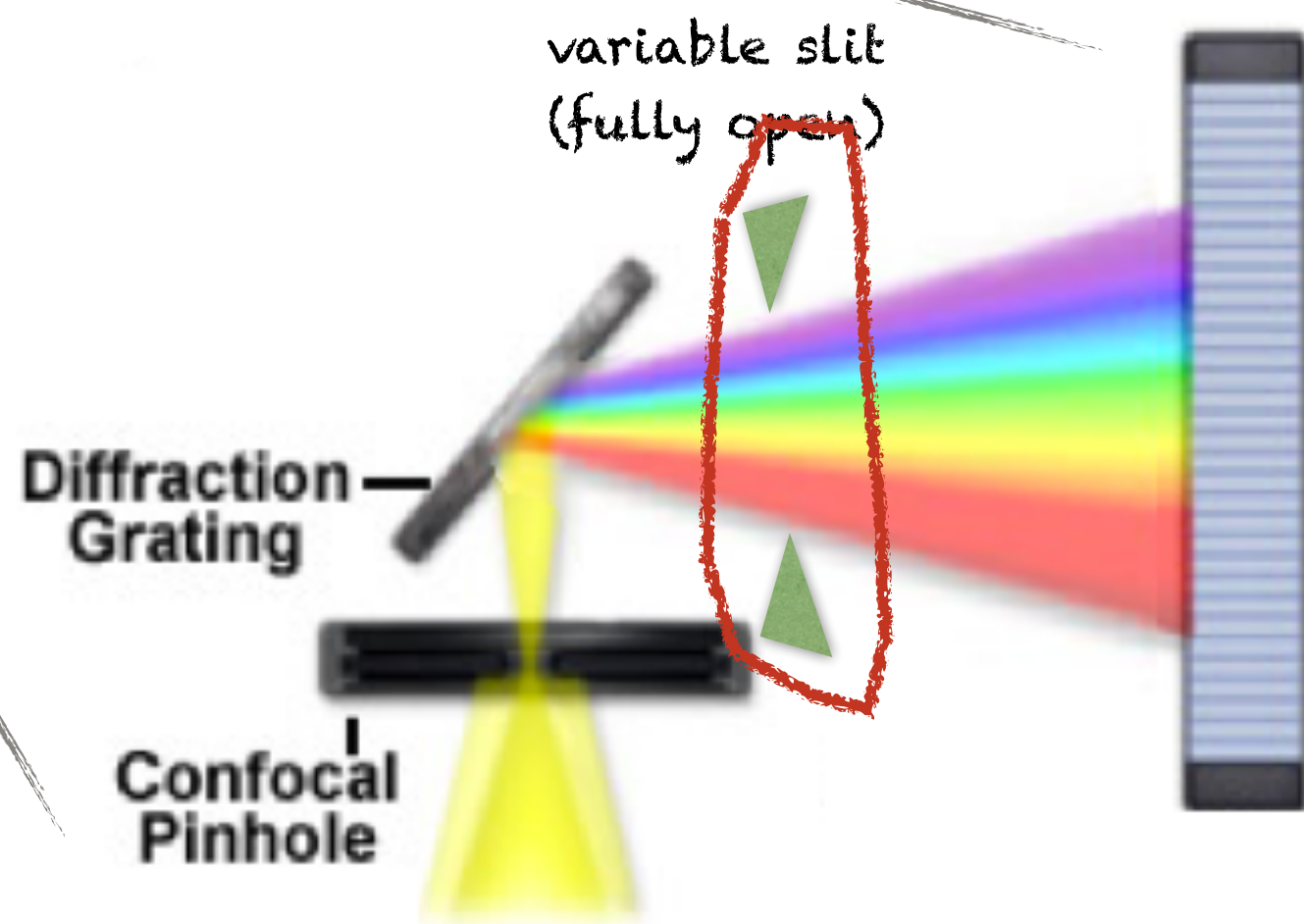
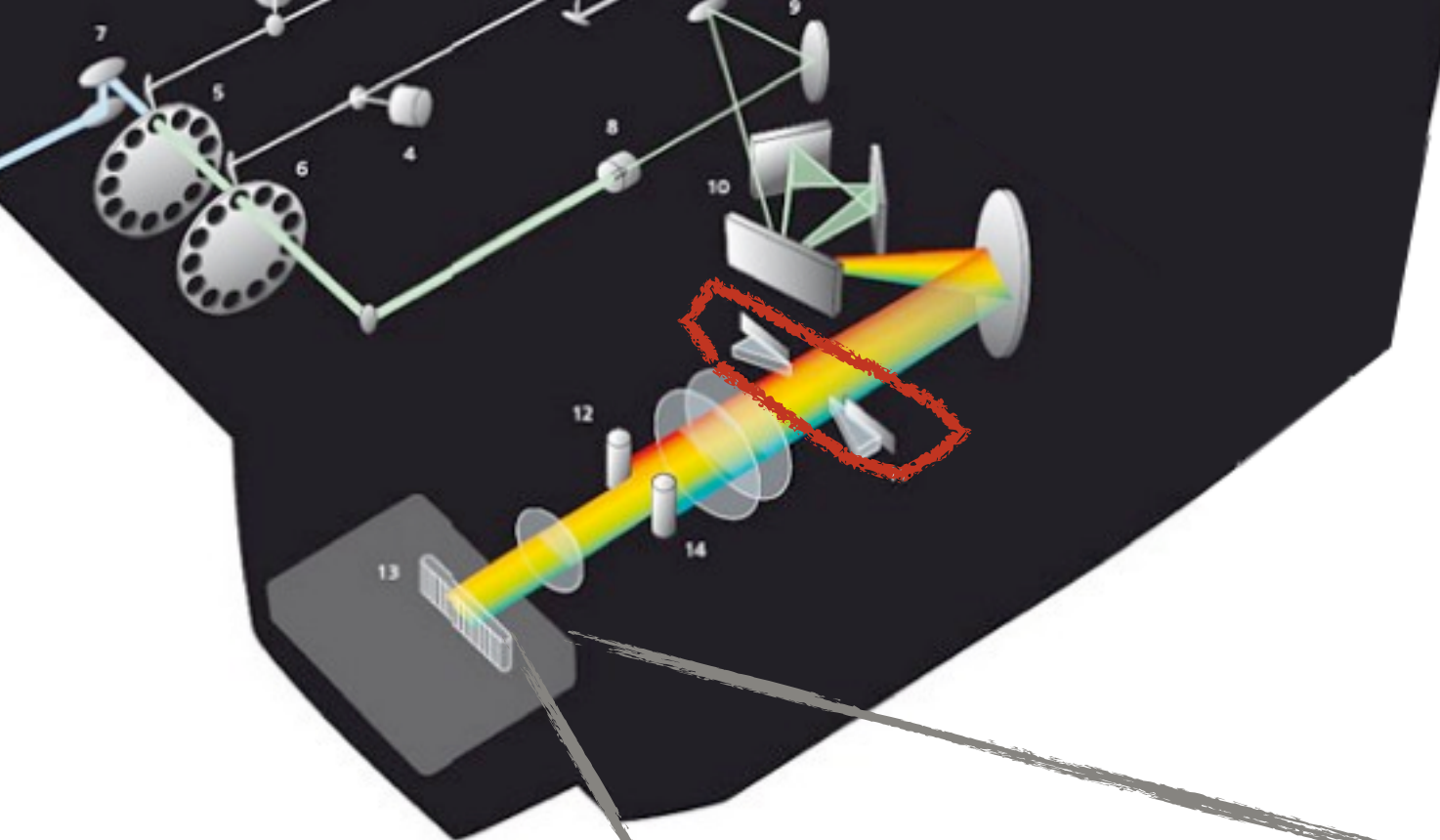
a row of PMTs #2-32 (is called ChS1 in our software)

these are actually rather cells than tubes

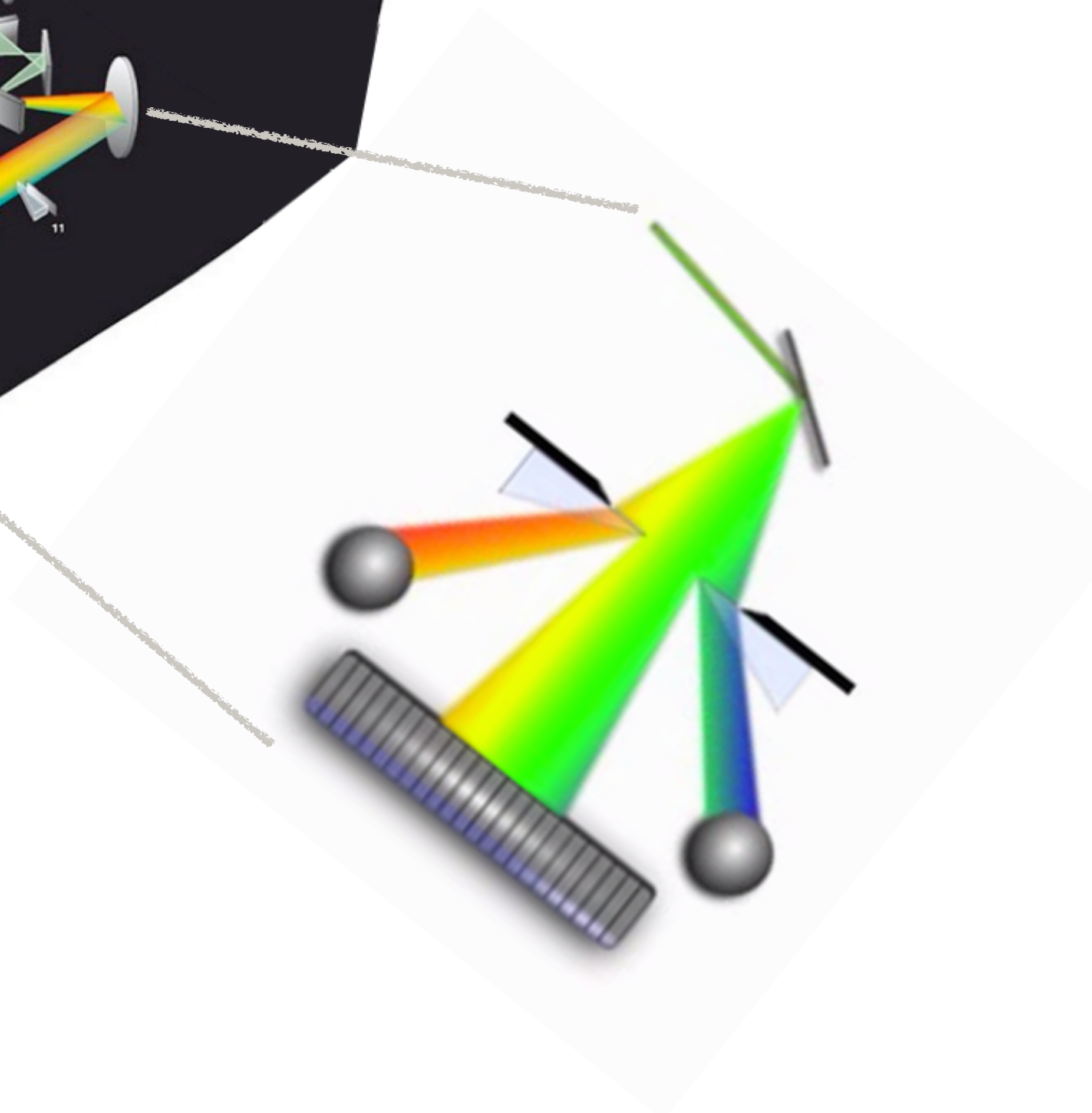
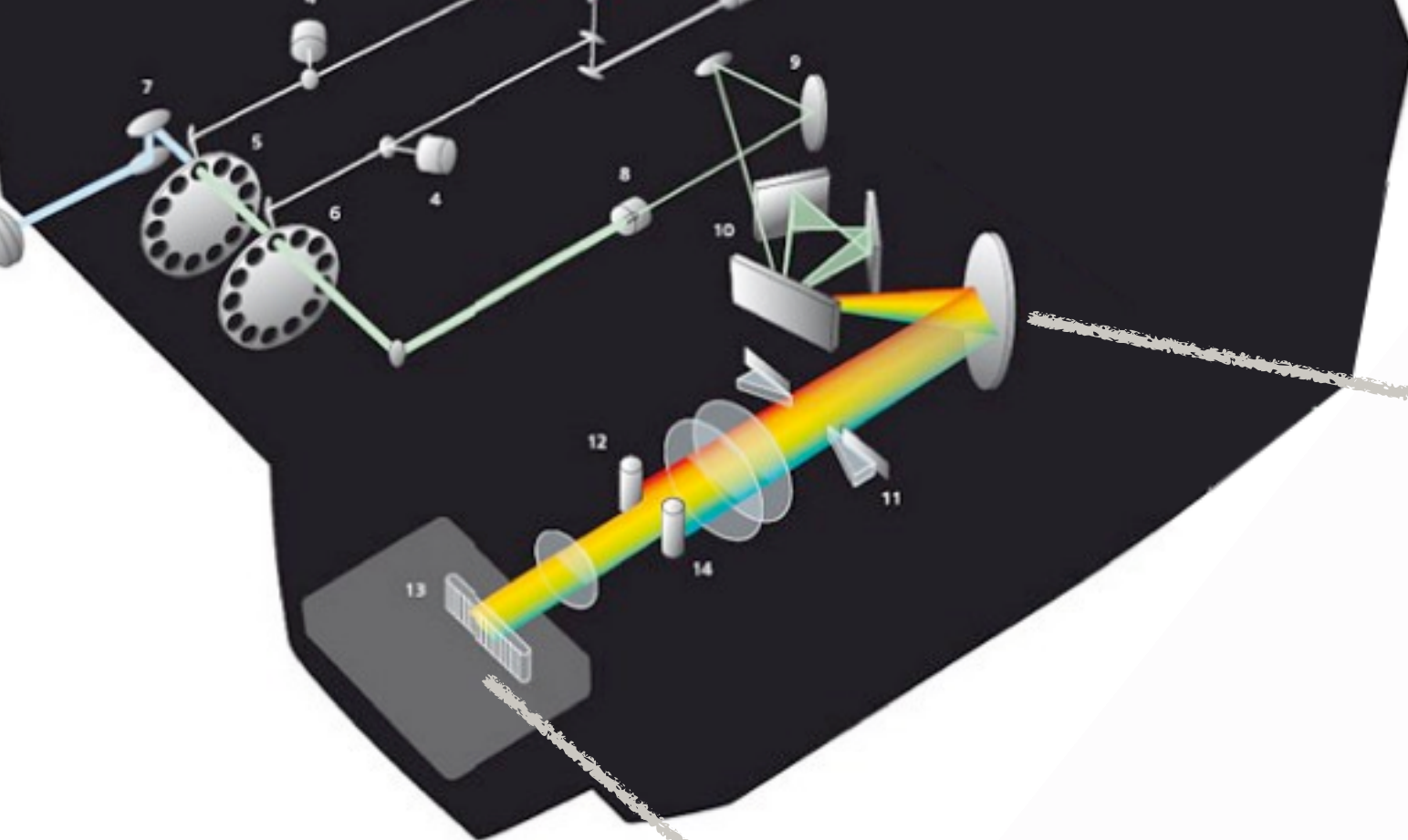






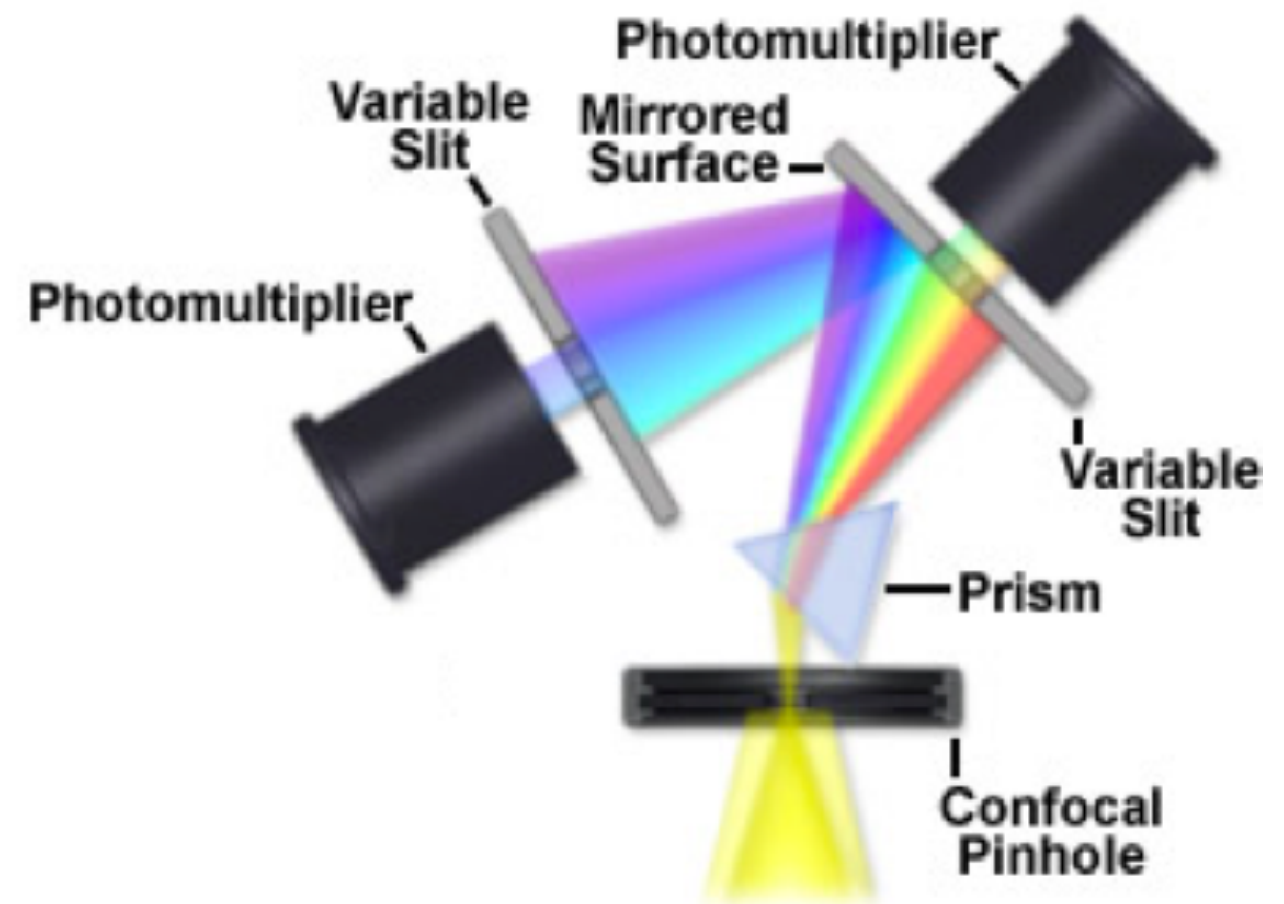
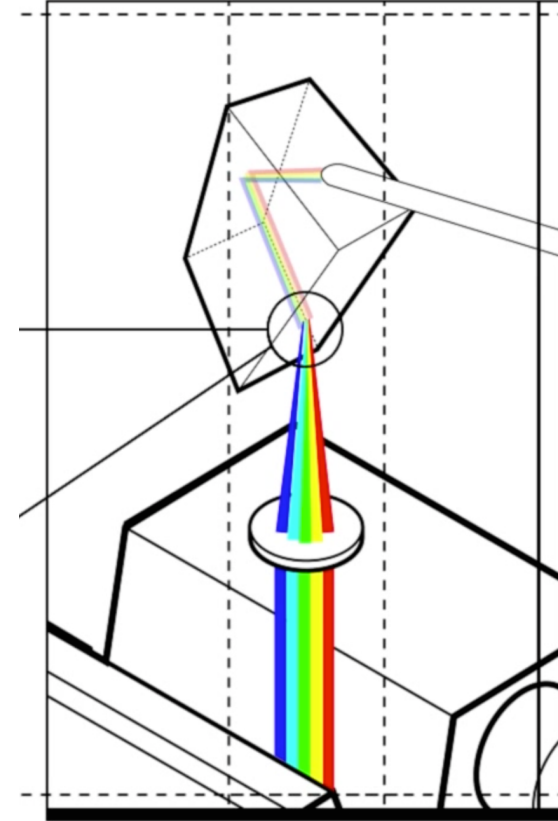
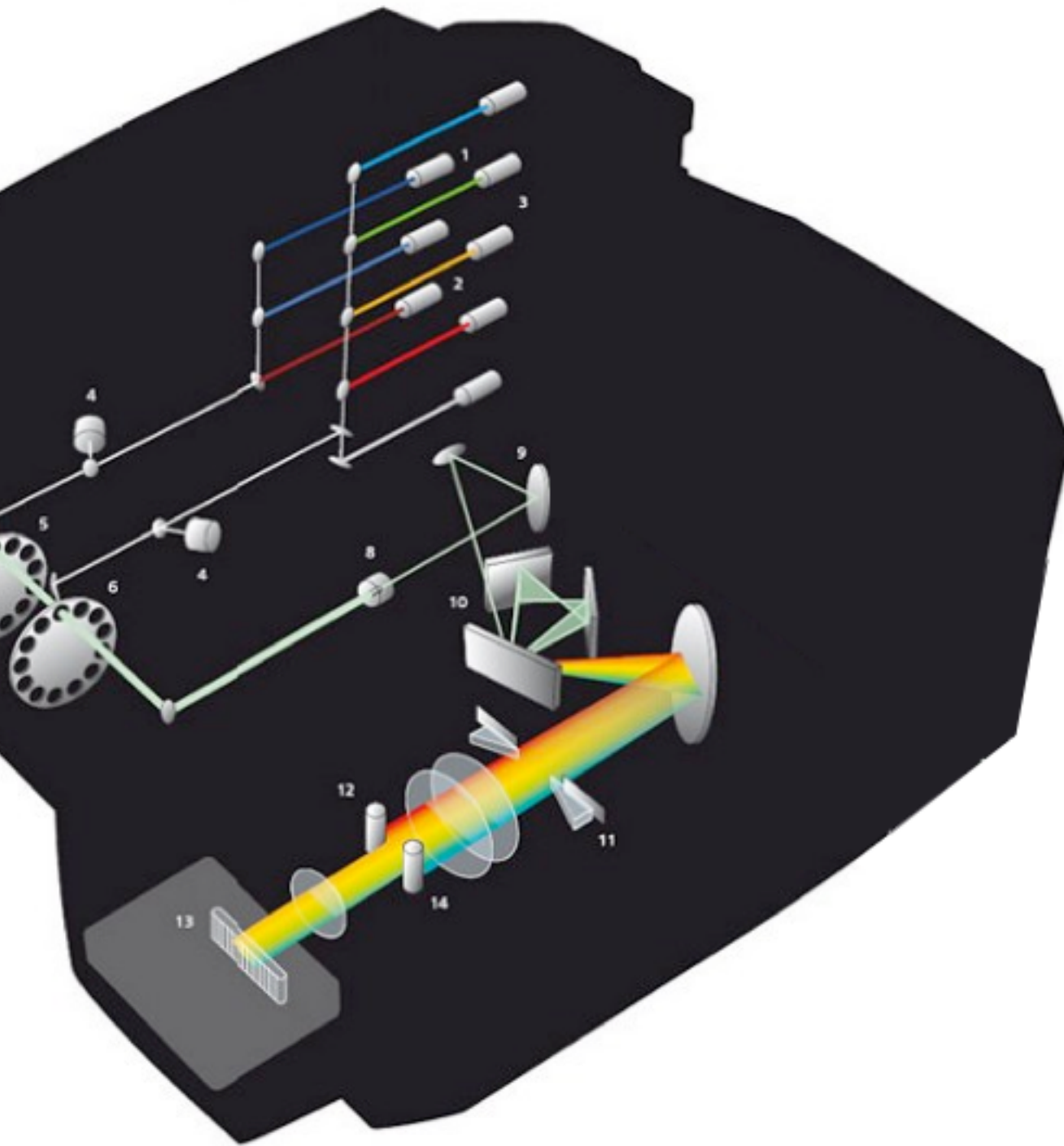








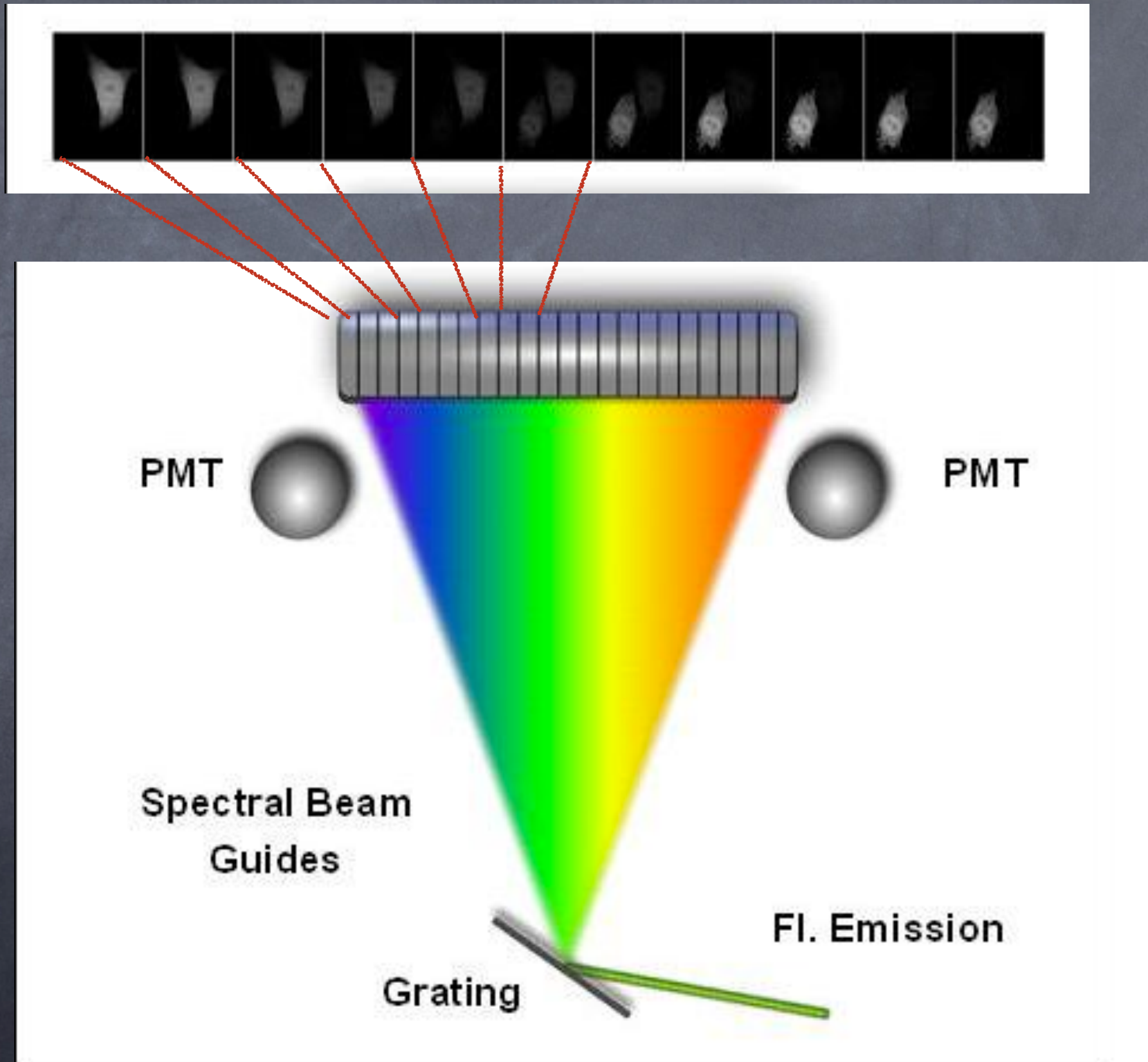
# Leica has alternative solutions





# Lambda scan

very nice, but what's the point of it?

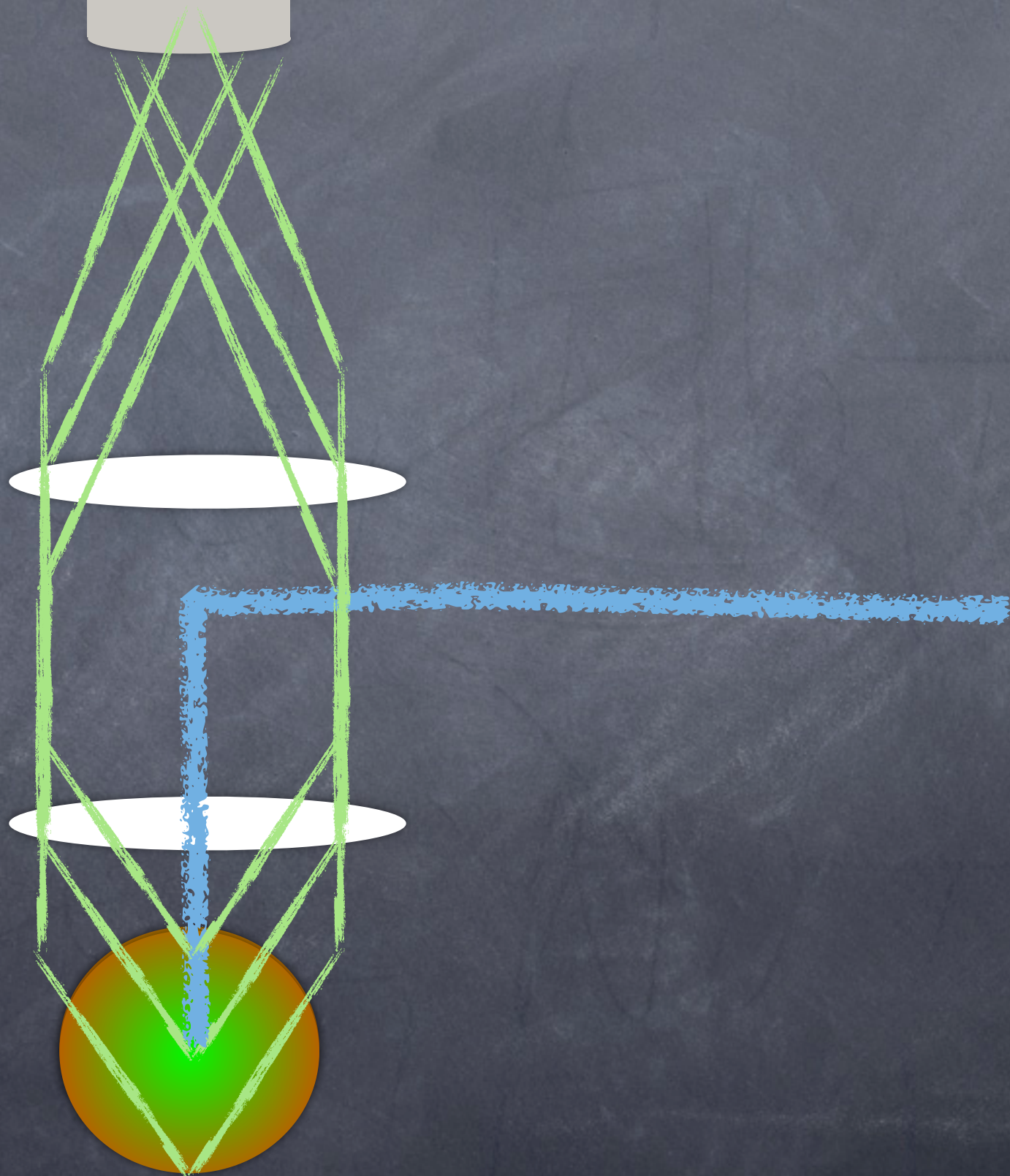




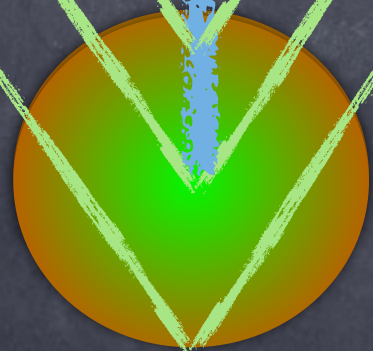
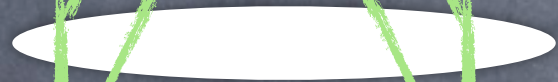
pinhole

a tiny whole of an adjustable size



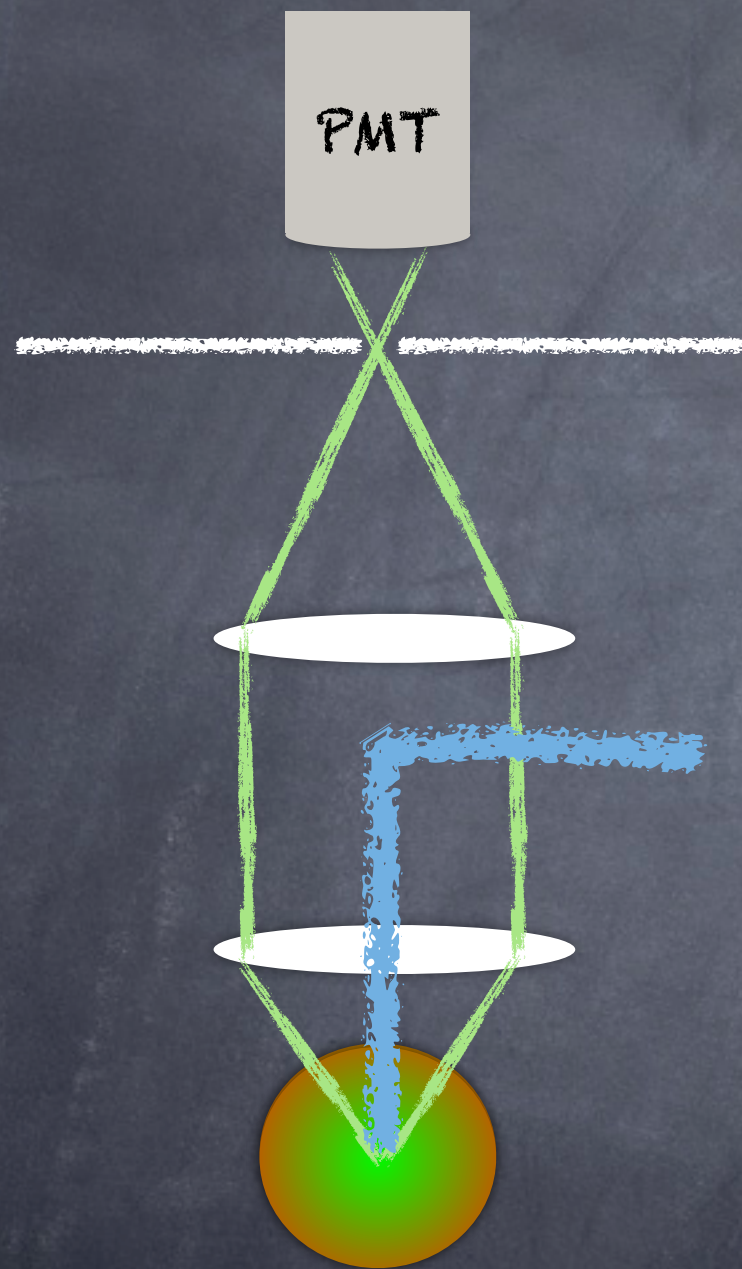








# The size of the pinhole



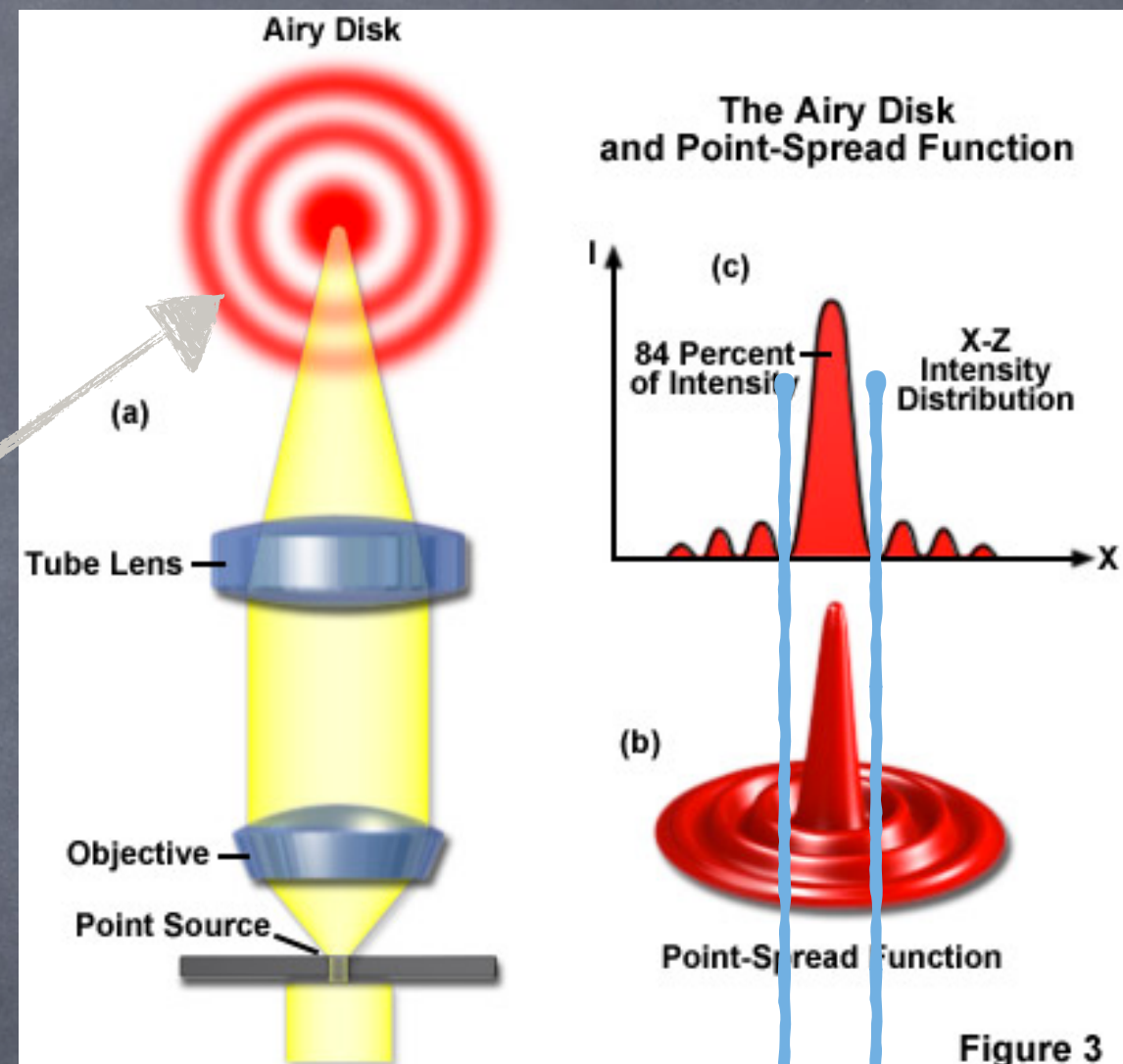
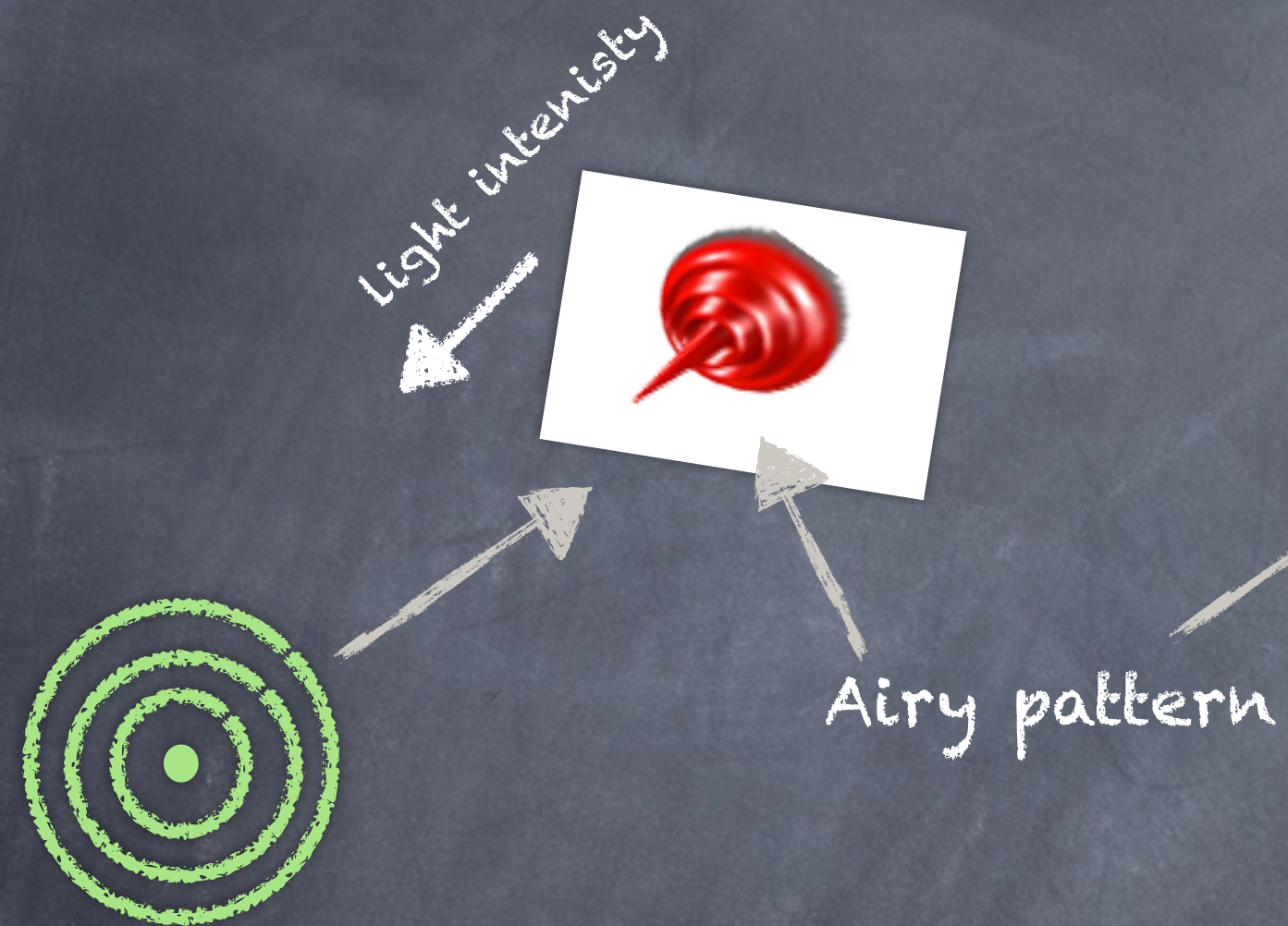
For best signal to noise ratio  
pinhole should match the Airy disk diameter





constructive/destructive interference  
of wavelets in the wave front will  
create an Airy pattern on the screen



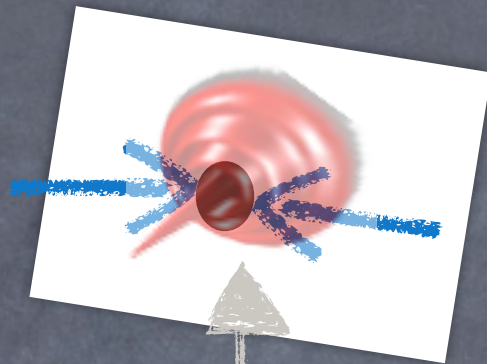
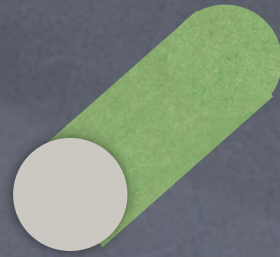


1 Airy Unit = 1AU = diameter of an Airy disk

$$1.22 * \lambda / NA$$

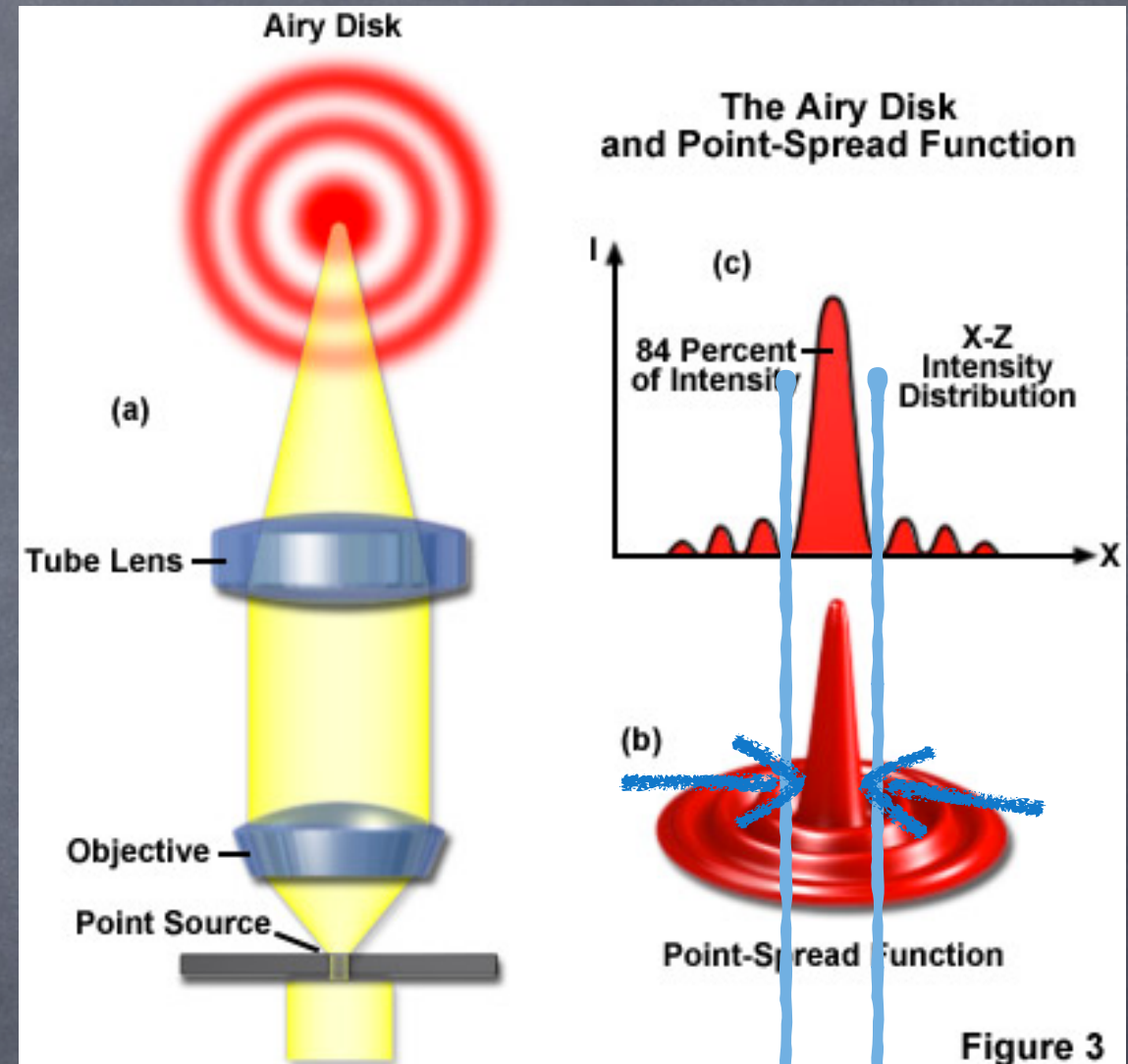


PMT



if there is a hole in the screen  
and its diameter matches the  
diameter of the airy disk  
most of the intensity will pass  
through it to the PMT detector

pinhole



1 Airy Unit = 1AU = diameter of an  
Airy disk

$$1.22 * \lambda / NA$$