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We use this protocol and it's working

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Insert Alignment ELYRA7

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DISCLAIMER

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ABSTRACT

Alignment of the stage insert to ensure that the sample is perpendicular to the optical axis. This protocol is based on material provided by ZEISS' application specialists.

What is needed before to start:
- This protocol was designed for the ELYRA7
- You will need 2 microscopy slides, one of standard size and one of about 2x2cm

Turn on the microscope

1 Turn on the ELYRA 7

1.1 Turn on **Main Switch**

1.2 Turn on **Components**

1.3 Turn on the Microscope Computer

> **Note**
>
> In the ELYRA 7 if the microscope computer is not on, then the microscope's touch screen will not light up.

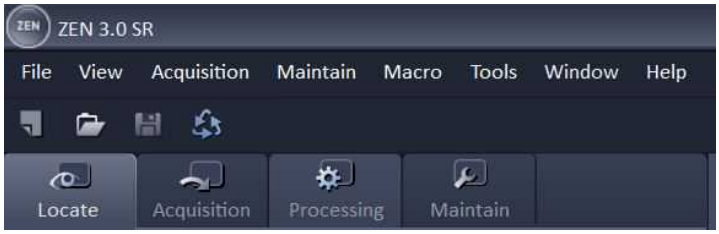
1.4 Open **ZEN black**, and choose *start system*

Find sample plane via the eye pieces

2

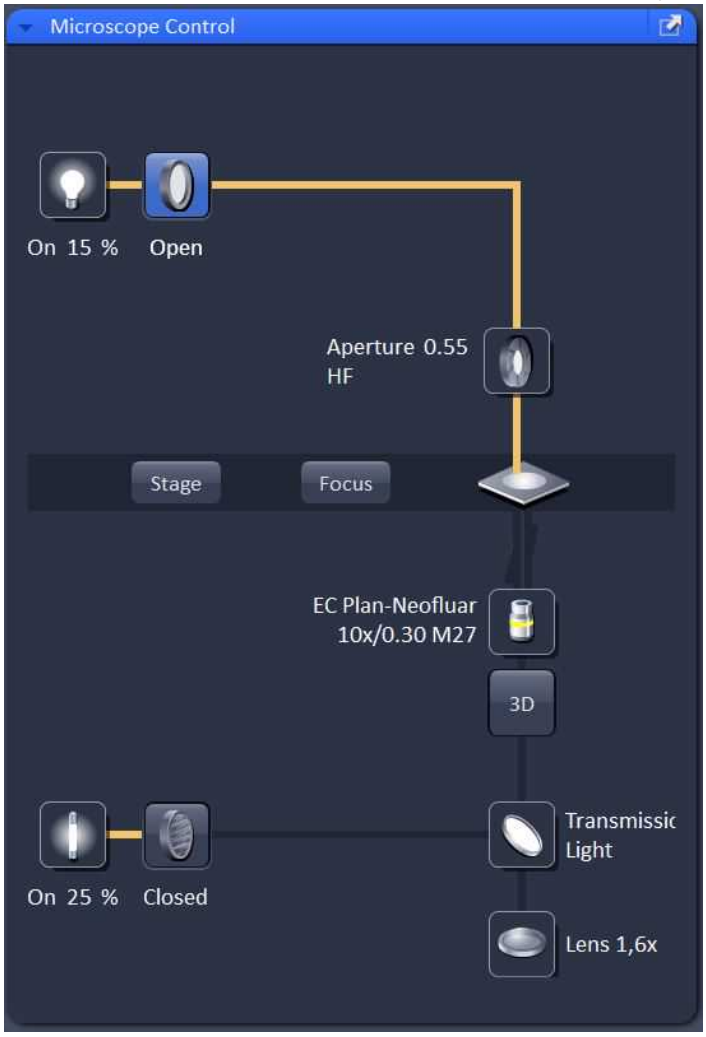
> **Note**
>
> This protocol assumes that the microscope is on, and ready for operation

ZEN black -> **Locate Tab,**



*View of **Tabs** on ZEN black, top left side*

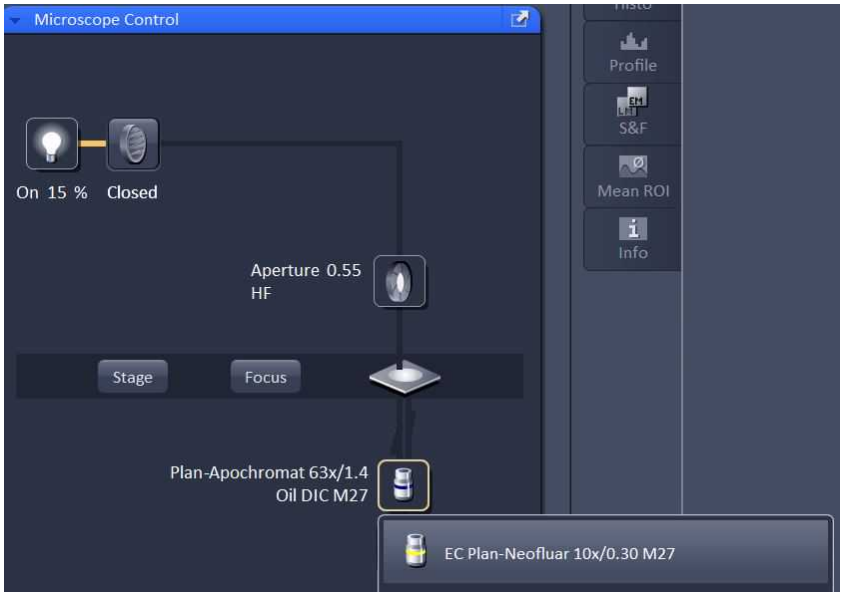
and set an imaging protocol for transmitted light so you can see the sample via the eye pieces.



Microscope Control example for transmission

Software	
ZEN black	NAME
ZEISS	DEVELOPER

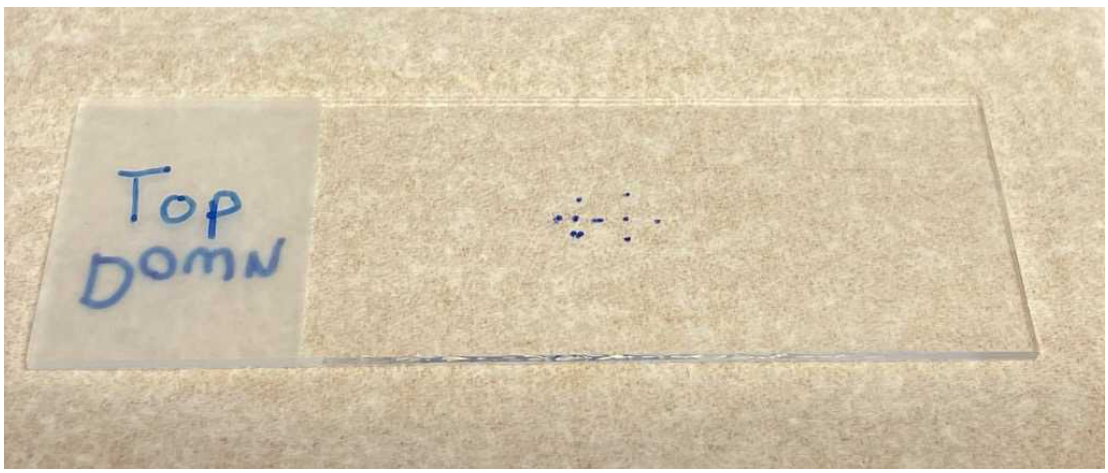
3 Change the objective to the lowest magnification, typically 10X



*You can change objectives in the **Microscope Control panel***

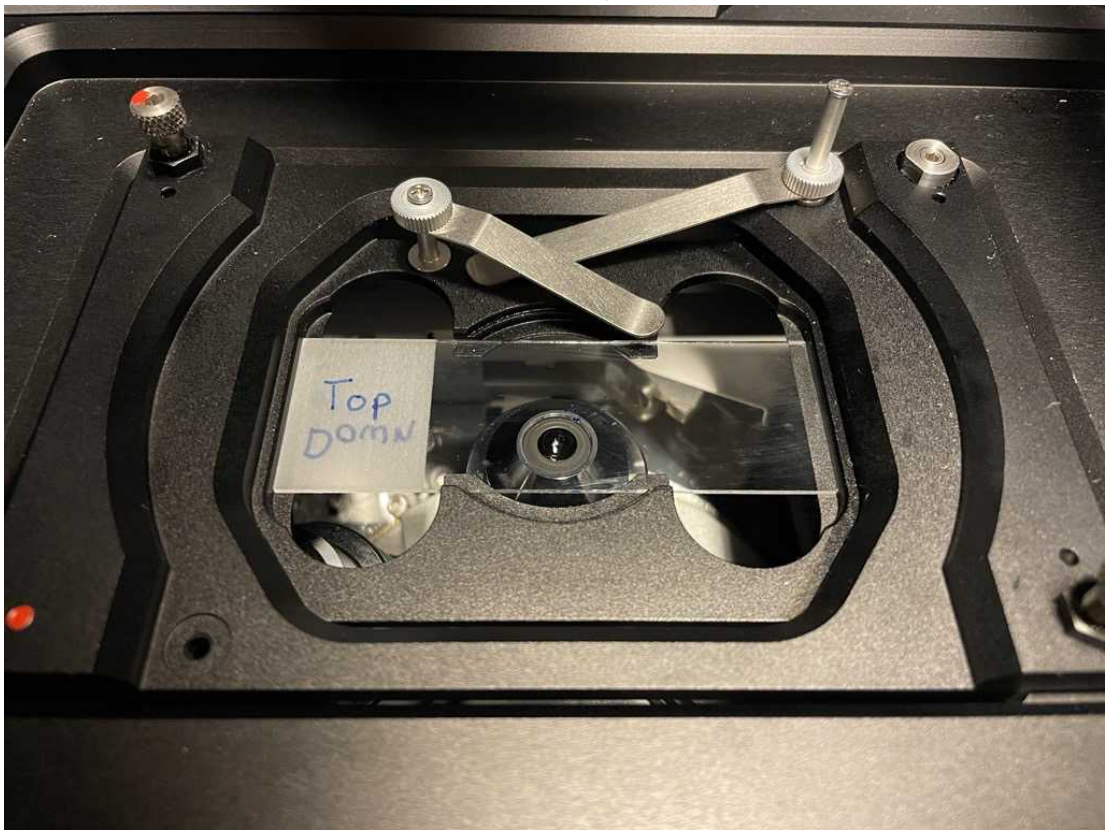
4 Prepare a clean microscopy slide having a few marker points, *marker-slide*. You can keep track of the surface on which you made the marks by writing TOP and DOWN

> **Note**
>
> The slide can be reused



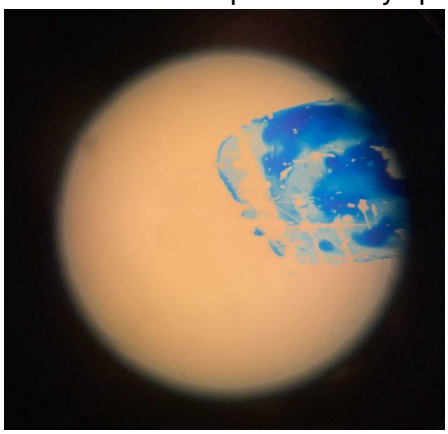
Example of marker-slide used to move to find the sample plane by focusing with the low magnification objective

5 Place the *marker-slide* on the sample holder so you can focus on the appropriate sample plane



Marker-slide on the slide holder

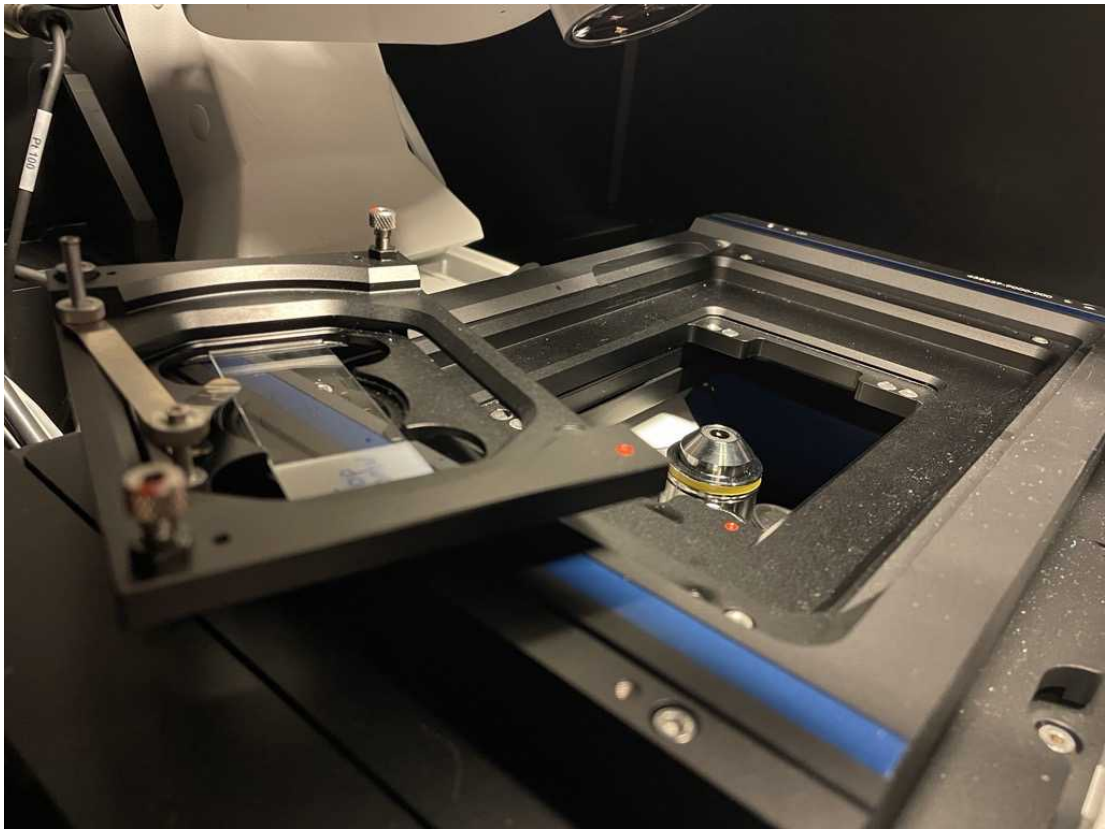
6 Focus on the sample via the eye pieces



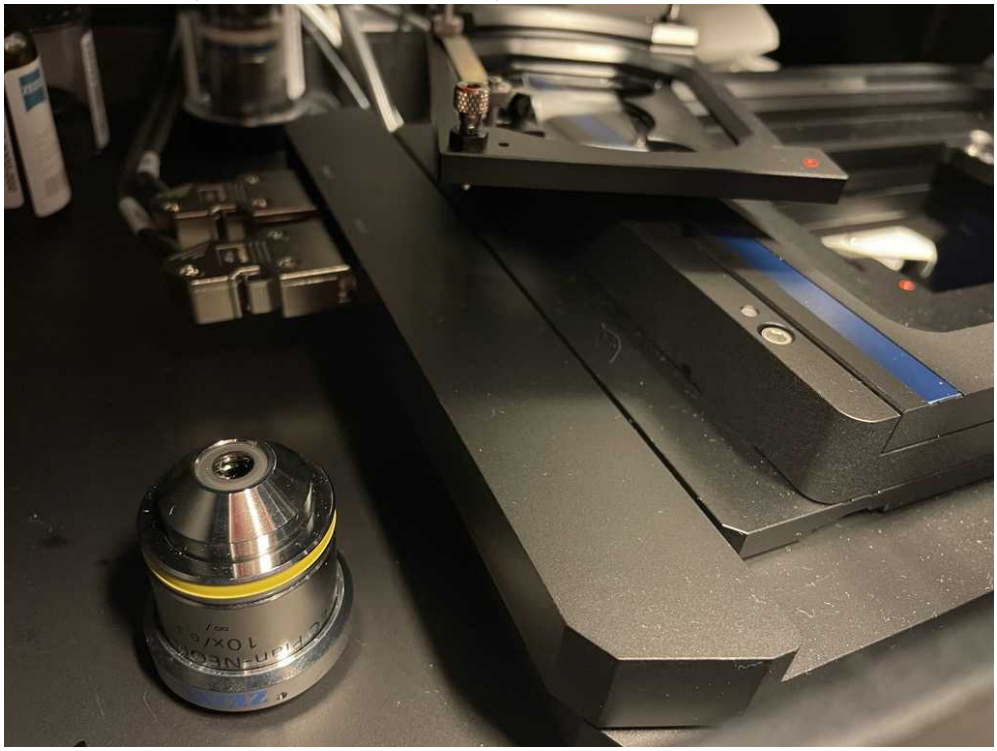
Marker spot in focus as viewed via the eye pieces

Place glasses for reflection mode

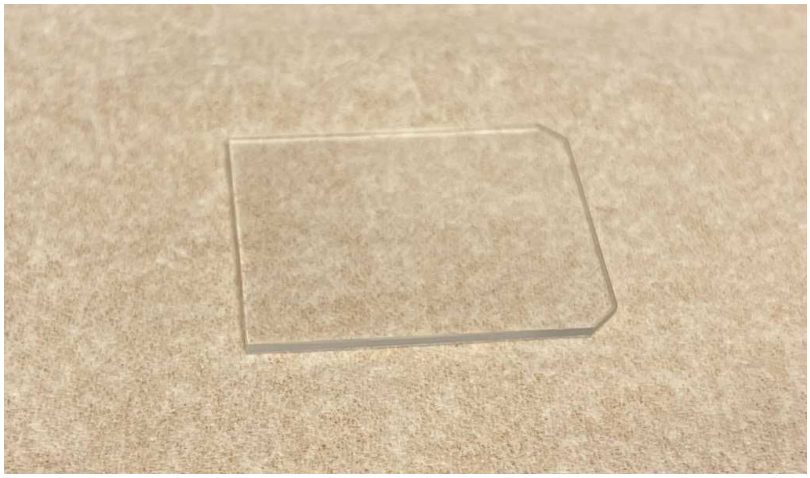
7 Remove the sample holder carefully and place it a side



8 Remove the objective lens and carefully place it a side



9 Prepare a clean microscopy slide of size aproximately 2x2cm

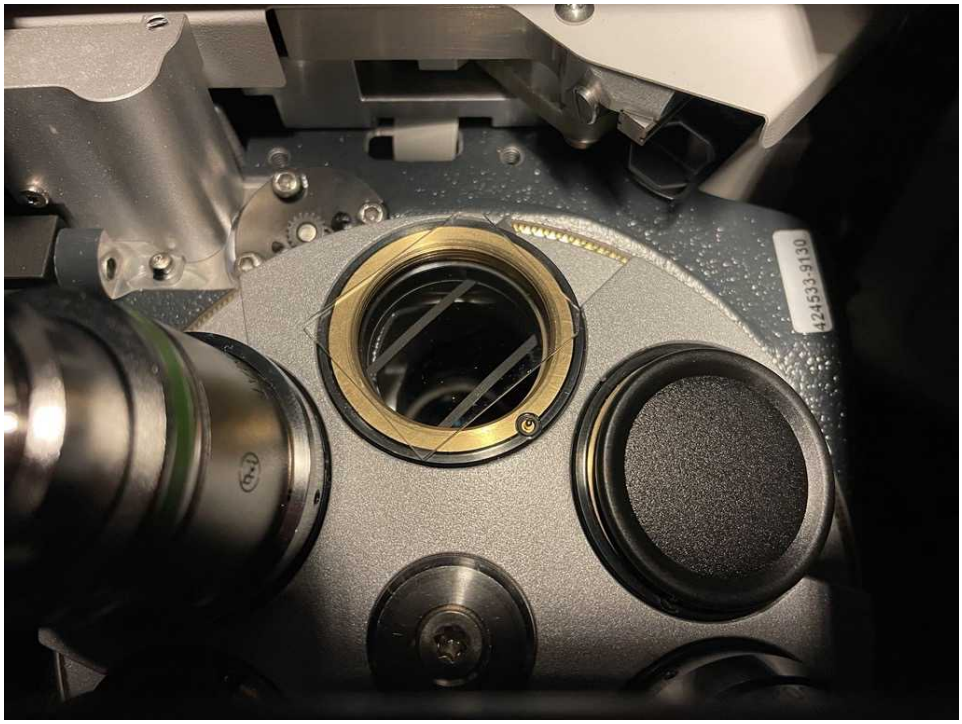


Small slide can be made by cutting a larger slide into the appropiate size

10 Place the 2x2cm clean glass plate on the objective turret.

Safety information

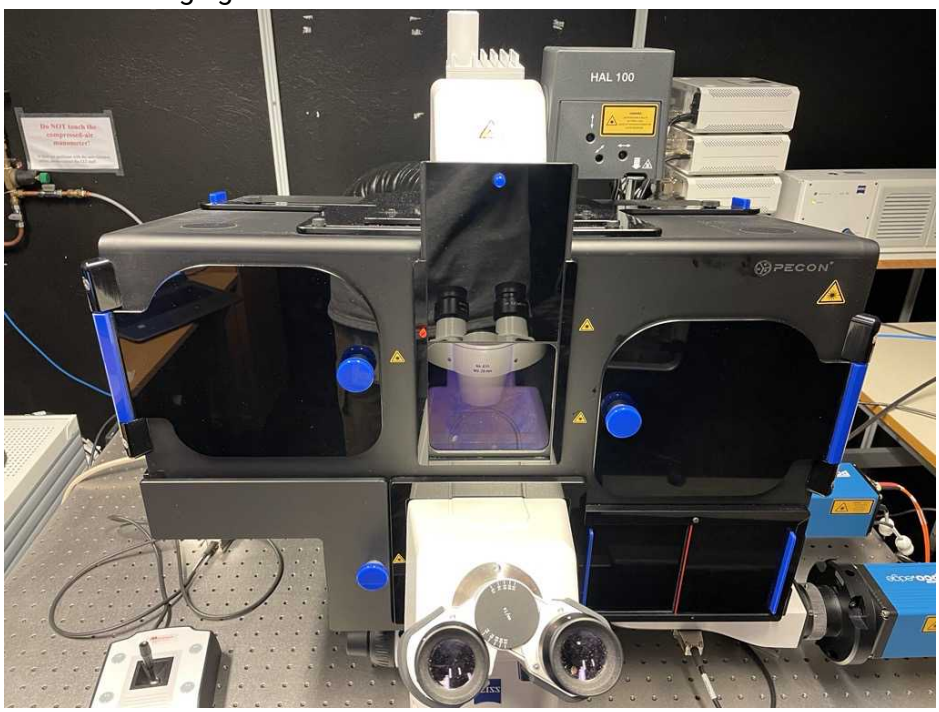
To avoid adding fingerprints to the slide you can wear protective gloves.
Make sure not to touch the cupper pin with with glass. This pin is used to read the objectives collar, and it is delicate.



Place back the sample holder (which still has on it the marker sample)

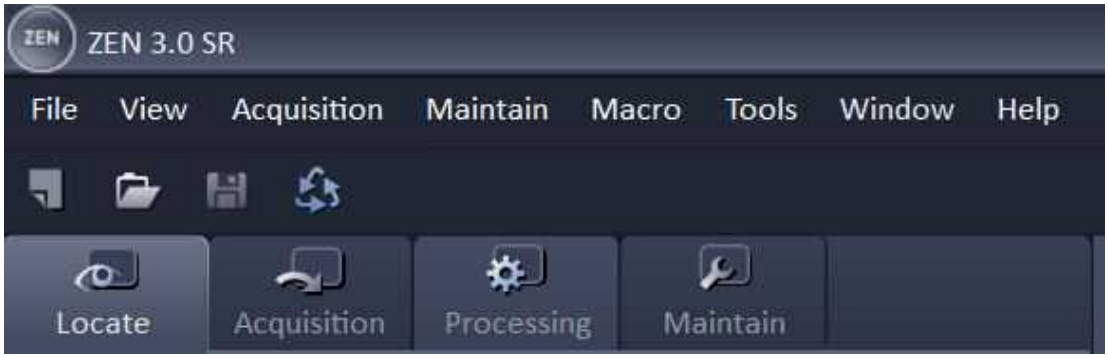
11

12 Close the imaging chamber



Acquisition Settings

13 Go to ZEN black -> **Acquisition Tab**



14 Set the aquisiton settings

Step 14 includes a Step case.
You have settings
You need settings

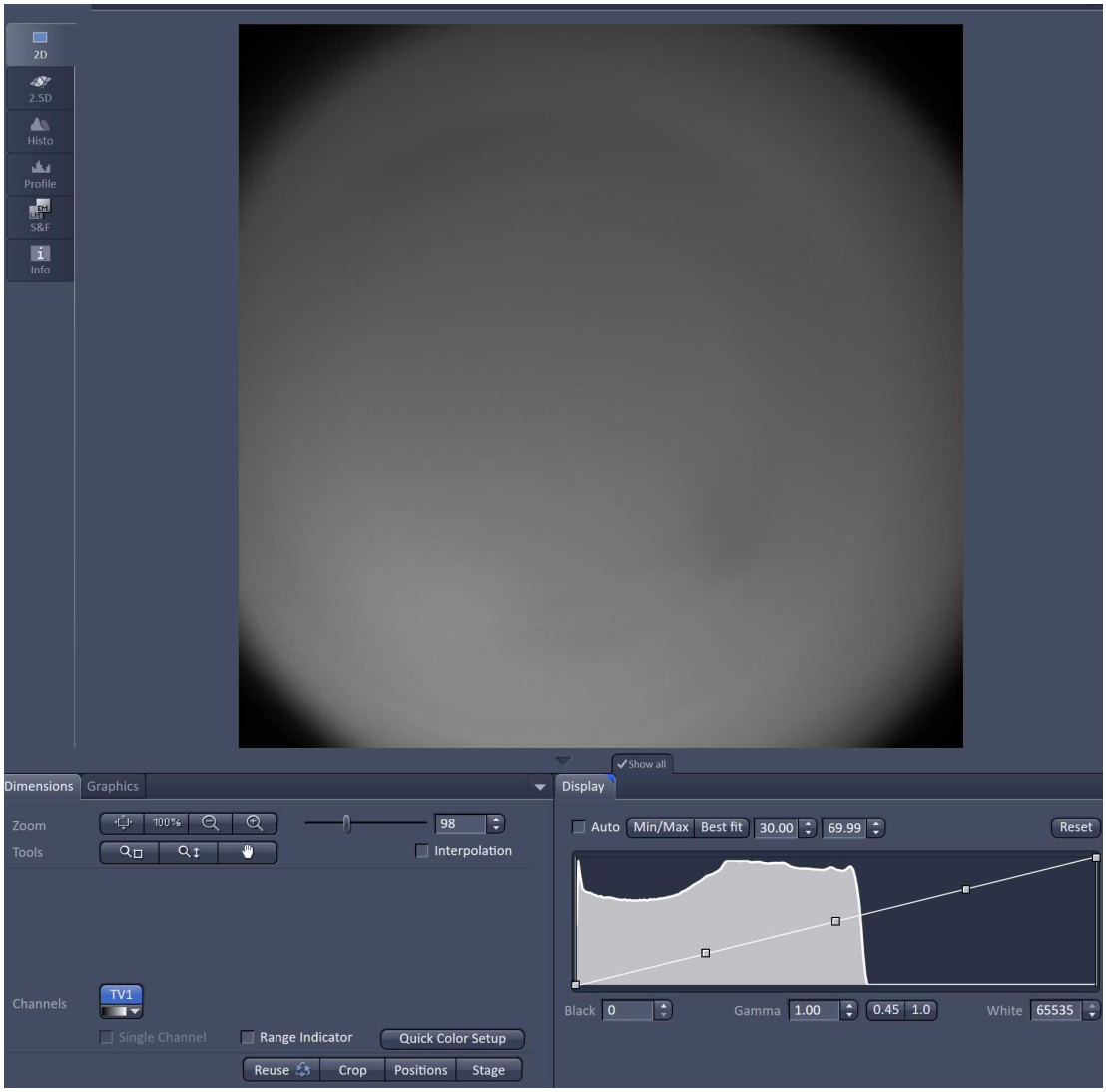
step case

You have settings

15 Load the acquisiton settings by using the **Experiment Manager**
In our case these settings are stored as "insert alignment"

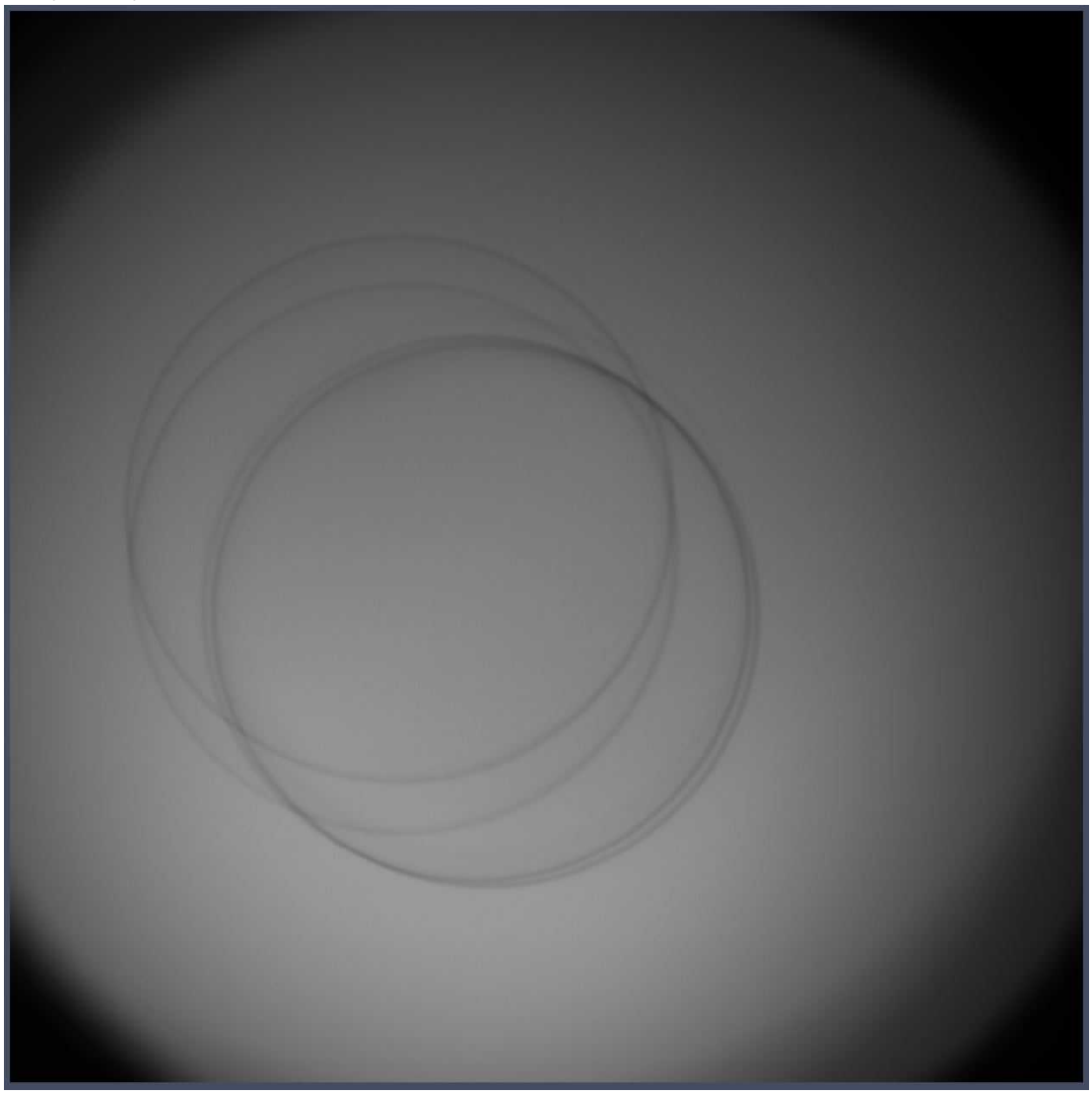
Image Acquisition

16 Go to **Live mode** and make sure image is not saturated

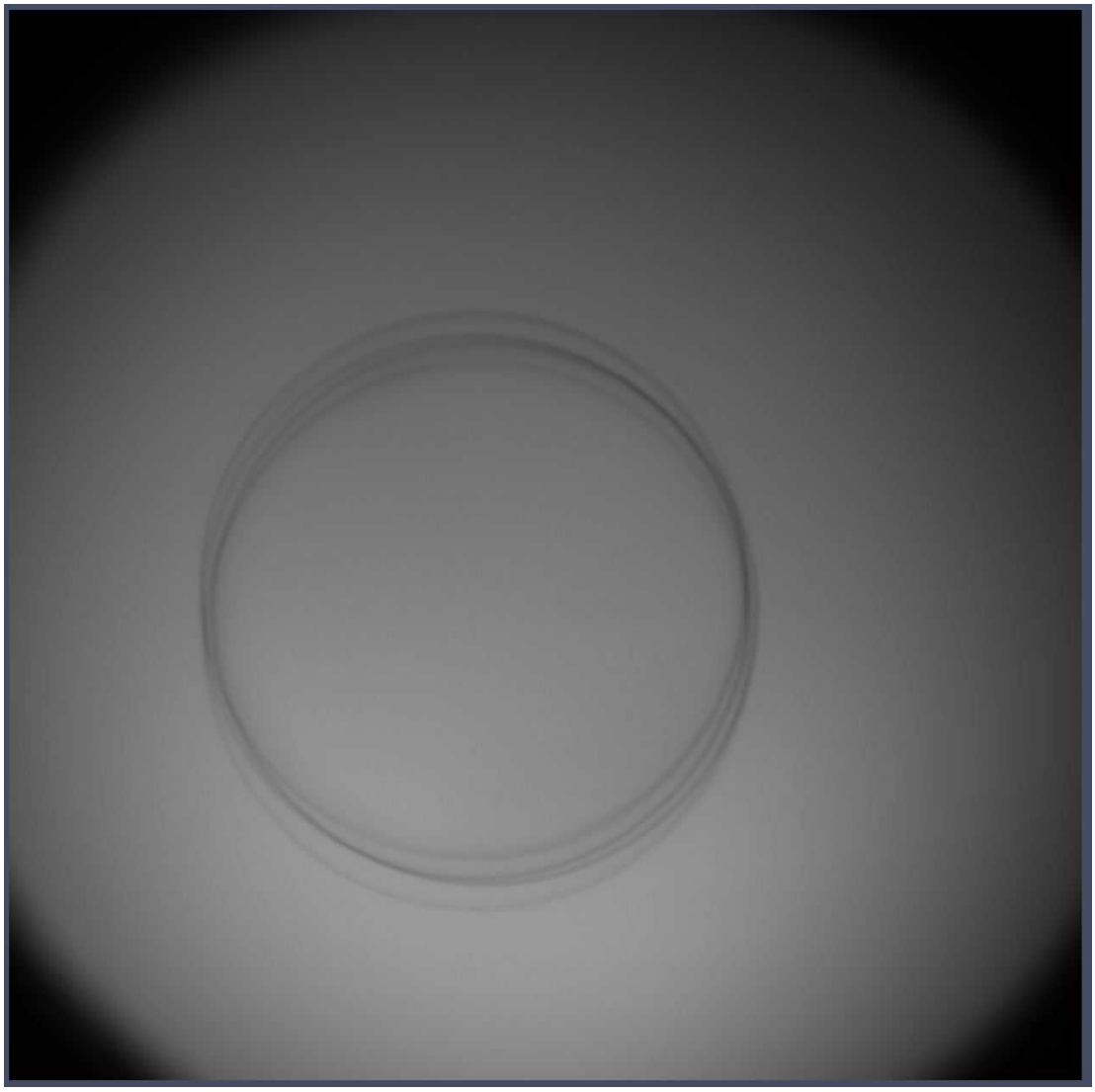


17 Find where the fibre of the HXP is connected to the microscope body. This will be on the left hand side of the microscope's body, after the on/off button

18 While in **Live mode**, carefully take out the fibre from the HXP that is connected to microscope body until you see the sharp edges of the fibre in the field of view



19 Use the levelling screws of the holder to match the 2 sets of reflections to one another



20 Take an image **snap**, and save the image for your own record

21 Place back the HXP fibre into position so you do not see the edges of the fibre while in **live** view

Place objective back into the turret

22 Remove the sample holder carefully so you don't touch/move the levelling screws

Note

From now on be very careful of not moving the levelling screws

23 Remove the glass slide placed at the base of the objective port

24 Place back the objective

25 Place back the sample holder

Safety information

Be careful not to touch the levelling screws and not to hit the objective lens with the holder.