OptArc x40 dry and x100 oil Objectives

User Guide

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Manual prepared by **OptArc** – Designers and manufacturers of custom optical and optoelectronic systems using 3D printing rapid prototyping and based in the UK.
See **optarc.co.uk** for details.

Safety Information

Identification of risk

Throughout this manual please take heed of **warnings given in bold text and highlighted yellow** to avoid possible damage to equipment and/or harm to people.

Risk to vulnerable groups

OptArc lab supplies and equipment are not toys. They contain small parts which may come loose, glass components that may splinter or break or otherwise present a choking or sharp object hazard or chemical hazard. **Please do not let babies or young children play with or gain access to any aspect of this product. Older children should only access this product with close appropriate adult supervision. Likewise keep this product away from pets**.

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Introduction

The OptArc high magnification objectives (x40 and x100 oil) allow fine morphological features to be seen at magnifications of x400 and x1000 when used with a x10 eyepiece.

Unlike the lower magnification objectives, **these lenses have a long casing that restricts the clearance of the specimen from the lens. This means that it is relatively easy for the specimen or slide to come into physical contact with the objective lens and this may cause damage to the specimen and/or the objective lens if not handled correctly**.

The purpose of this manual is to provide the necessary guidance to minimise the risk of adverse consequences to the sample and objectives by describing safe methods of use.

These issues are also relevant regardless of the supplier of the objective lens – even if you are using lenses that are not supplied by OptArc. Also, even if you are an experienced microscopist with traditional bench top laboratory microscopes, the unique design of the PUMA microscope poses its own challenges. So please read this manual fully before first fitting or using any high magnification objective lens with a PUMA microscope

Need for a condenser lens

The x40 objective has a numerical aperture (NA) of 0.65 and the x100 oil has a maximum NA of 1.25. These objectives will, therefore, only give good quality images if they are used with a wide angled beam illumination system. Without such illumination the images will appear ‘tinny’, hypercontrasted, dark, overly refractile and lacking in resolution.

Such an illumination system is conventionally achieved by means of a condenser lens.

For transillumination the PUMA Abbe condenser is recommended (figure 1).

For co-axial epi-illumination the objective lens itself will act as a well-matched condenser system.

The full Köhler illuminator is not a pre-requisite but it will provide good quality full-field flat trans-illumination when used in conjunction with the Abbe condenser

For more detail about condenser lens systems see the following videos on the PUMA microscope YouTube channel:

Figure 1. The optional PUMA Abbe condenser upgrade is recommended for use with high magnification objectives.

Abbe condenser video

https://youtu.be/2wpsvA2cQgQ

Epi-illumination video

https://youtu.be/cAEB10K8PqI

Köhler illumination video

https://youtu.be/XEE-el7vC5k

How to insert and remove a specimen for use with the x40 (dry) objective

Because the PUMA microscope does not have a swing out objective turret and space on the stage is very limited (compared to a conventional bench-top microscope), **there is a danger that inserting a slide onto the stage with the objective in place could result in an edge or corner of the glass slide coming into contact with the front lens of the objective and scratching it.** This will permanently damage the objective and cause loss of image clarity. A non-coverslipped slide or specimen has an even greater risk of the specimen contaminating or scratching the lens if it comes into contact.

For that reason the recommended procedure for inserting and removing a **cover-slipped glass slide** when using a high magnification objective is as follows:

1. With a low power lens in place (like the x4 lens that comes with a Foundation scope kit), insert your slide onto the stage and focus the microscope.

2. Move the slide till the the area of interest is in the centre of the low power field of view.

3. Raise the focus platform by two full rotations of the fine focus gear (ensure you are raising it – to increase the distance between the slide on the stage and the objective fixed to the focus platform – not lowering it – see figure 2).

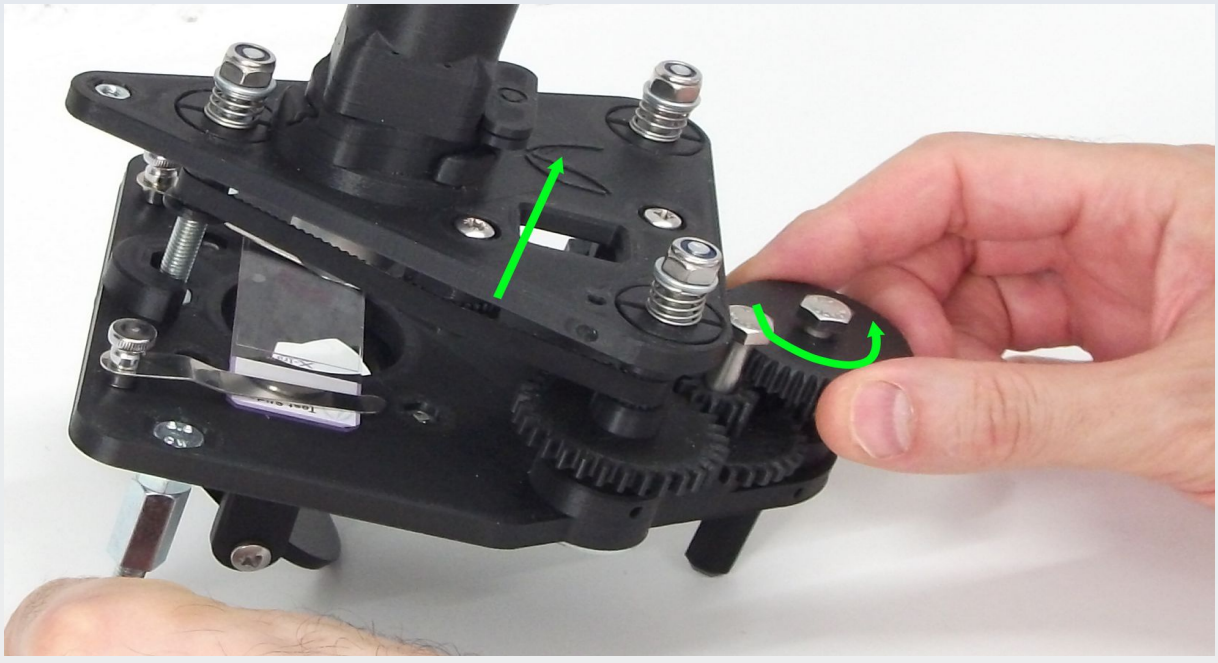

Figure 2. Turn the fine (or coarse) gear in the anti-clockwise direction to raise the focus plate and lift the objective away from the specimen on the stage.

4. Remove the optical tube from the microscope and change the objective to your high power objective. Ensure it is screwed in fully to the RMS thread.

5. Re-insert the optical tube. When fully inserted look closely from the side to ensure there is still a gap between the front lens of the objective and the slide on the stage. If there is no gap then you should raise the focus plate till you see at least a 1 mm gap (see figure 3)

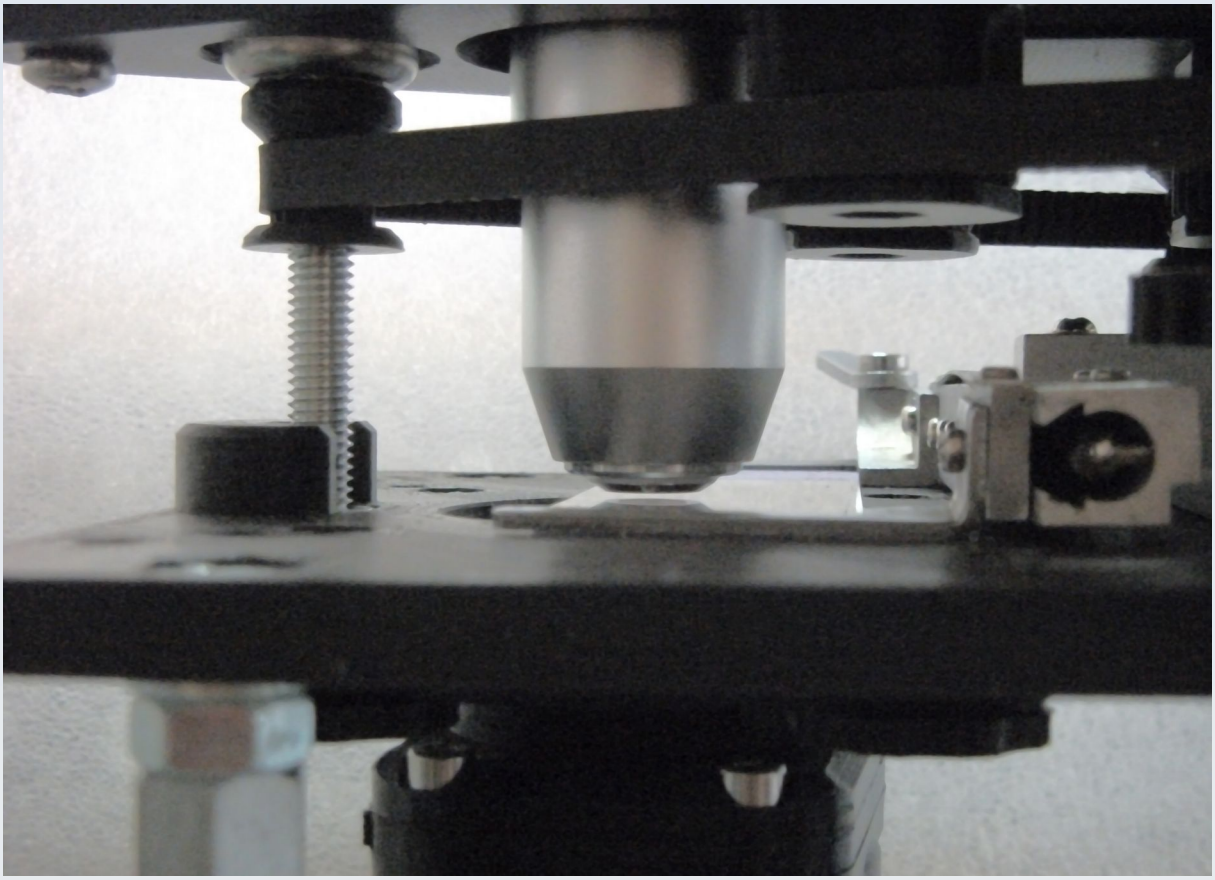

Figure 3. Looking at the x40 objective from the side, the slide will be in focus when the working distance (the gap between the lower lens of the objective and the top surface of the coverslip) is about 1 mm, as shown. This is also about the same distance as the pitch of the thread in the focus post bolt (seen on the left).

You can now focus the microscope with the fine focus wheel while looking down an ocular lens till the specimen is in focus. If you find you are making excessive turns of the fine focus gear (more than two full turns) then look again at the gap between the objective and the slide to ensure you have not gone too far down and made contact with the slide. If you have made contact then raise the focus platform with the focus gear until you get at least (about) 2 mm clearance between the objective and the specimen and try again. Ensuring that some contrasting feature is present in the field of view will assist the focussing endeavour. Another potential pitfall is that the focus platform gets stuck so does not lower when you turn the focus gear. See the video on troubleshooting the Z stage for more information about that and how to fix it:

https://youtu.be/ffLx28N85u

While the coverslipped specimen is in focus you can carefully slide the specimen around the stage but be very careful not to raise it, especially when the edge of the sample of a coverslip is near the objective lens, to avoid scratching the lens.

I**f your sample is not cover-slipped** specimen then even more caution is required to avoid contact between the specimen and the objective lens. In this cases I recommend several (more than 4) full rotations of the fine gear to raise the focus platform in step 3 above and careful direct vision from the side when re-inserting the optical tube in step 5 (stop and remove the tube if it appears that the objective may be coming close to making contact with the sample). Also **if moving a non-coverslipped specimen around on the stage take care than any elevated parts do not crash into the lens** (like components on a printed circuit board) – careful frequent observation from the side while moving the sample around is required.

The x40 objective is a dry objective. **You must not get any immersion oil or water or other substance onto the lens surface of this objective**. If you are going to observe a specimen with this objective that has just been observed with a x100 oil objective, be sure to first wipe and clean off any oil that remains on the surface of the specimen from your previous observations before putting the x40 lens in place. See the 'Maintenance' chapter for more about how to look after your objective.

To remove a specimen after completing observations with the x40 lens use the following procedure:

1. First ensure there is good clearance between the objective and the specimen (by looking from the side). This should already be the case if you were making observations in focus for coverslipped slides but if you have been observing non-coverslipped specimens like a PCB then elevating the focus platform to provide extra clearance from surrounding objects may be a wise precautionary measure at this stage.

2. Remove the optical tube from the scope

3. Remove the specimen from the stage.

How to insert and remove a specimen for use with the x100 (oil immersion) objective

Because the PUMA microscope does not have a swing out objective turret and space on the stage is very limited (compared to a conventional bench-top microscope), **there is a danger that inserting a slide onto the stage with the objective in place could result in an edge or corner of the glass slide coming into contact with the front lens of the objective and scratching it.** This will permanently damage the objective and cause loss of image clarity. A non-coverslipped slide or specimen has an even greater risk of the specimen contaminating or scratching the lens if it comes into contact. For oil immersion objectives this risk is even greater than with dry objectives (like the x40) so **we recommend that you only use oil immersion (or specialist water immersion) objectives with coverslipped specimens.**

The recommended procedure for inserting and removing a **cover-slipped glass slide** when using a x100 oil immersion objective is as follows:

1. With a low power lens in place (like the x4 lens that comes with a Foundation scope kit), insert your slide onto the stage and focus the microscope. If you are going to oil the PUMA Abbe condenser for maximum benefit, first put one or two drops of immersion oil on the top lens of the condenser then insert your slide onto it to get a layer of immersion oil spreading between the undersurface of your slide and the top lens of the condenser. Do not put any oil onto the coverslip at this stage. Please note, not all condensers are suitable for oiling in this way. The PUMA Abbe condenser is suitable by design. If you are using any other condenser please check with its specifications as to whether it may be oiled. Do not oil a condenser that is not designed for oiling or oil may get inside the lens system and damage it. For the PUMA Abbe condenser use only one or two drops of oil and be sure to wipe excess oil off the condenser after the slide has been removed at the end of your imaging session.

2. Move the slide till the the area of interest is in the centre of the low power field of view.

3. Raise the focus platform by two full rotations of the fine focus gear (ensure you are raising it – to increase the distance between the slide on the stage and the objective fixed to the focus platform – not lowering it – see figure 2).

4. Remove the optical tube from the microscope and change the objective to your high power objective. Ensure it is screwed in fully to the RMS thread. I recommend that you first focus the scope with a dry high power objective if you have one (such as the x40 objective) following the procedure for inserting and focussing the x40 dry objective from step 4 onwards as described in the section on ‘How to insert and remove a specimen for use with the x40 (dry) objective’ above. This helps you centre in on whatever high power field of view you want to examine by oil because it will be unlikely that you will be able to see such detail with a low power x4 objective). This also gets the scope into a better par-focal situation so you will not be far out of focus if you first focus with a x40 dry objective before switching to the x100 oil. Once you have located you field of interest with the dry x40 objective, raise the focus platform by two full rotations and remove the optical tube. Replace the dry x40 lens with your oil immersion x100 lens.

5. With the optical tube removed, place a drop of synthetic microscope immersion oil onto the coverslip in the centre of the field of view. **Do NOT put oil directly onto the objective lens** (the lens will eventually 'dip in' to the oil on the coverslip as described below but you do not apply the oil directly to the lens). Do not use excessive amounts of oil. Literally one or two drops is all that is required.

6. Re-insert the optical tube containing your x100 oil lens. When fully inserted look closely from the side to ensure the front of the lens makes contact with the oil on the slide coverslip. If it has not made contact even after ensuring the optical tube is fully inserted, this is OK, it just means you will need to first lower the focus platform with the fine focus gear till contact with the oil is made. You should also ensure that the objective spring-loaded mechanism has not been activated – if it has then the objective is too close to the specimen and your first focussing action must bee to raise the focus platform till the objective is lifted slightly off the coverslip.

You can now focus the microscope while looking down an ocular lens till the specimen is in focus. If you find you are making excessive turns of the fine focus gear (more than two full turns) without achieving focus then look again from the side at the gap between the objective and the slide to ensure you have not gone too far down and activated the spring retraction mechanism, or too far up and separated the objective lens from the oil layer. Ensuring that some contrasting feature is present in the field of view will assist the focussing endeavour.

While the specimen is in focus you can carefully slide the specimen around the stage but be very careful to to raise it, especially when the edge of the sample of a coverslip is near the objective lens, to avoid scratching the lens.

To remove a specimen after completing observations with the x100 oil lens use the following procedure:

1. First ensure there is good clearance between the objective and the specimen (by looking from the side). You should elevate the focus platform by a few turns to provide extra clearance from surrounding objects.

2. Remove the optical tube from the scope but **take great care to keep the objective lens in its working upright position at all times. Do not let it lay on its side or turn it upside down or immersion oil may drip and ooze into its protective spring mechanism and casing. At this stage, therefore, you should have a clean lens tissue handy to wipe away excess immersion oil from the front lens and surrounding metal before putting the optical tube down.**

3. Remove the specimen from the stage.

Remove the objective from the optical tube and continue to wipe clean the front surface of the objective with a lens tissue before keeping it safe in its supplied case prior to next use.

See the 'Maintenance' chapter for more information.

Maintenance

Immersion medium

The x40 objective must be used dry without an immersion medium of any kind. If any immersion medium gets onto the lens you should clean it off before further use. See 'Cleaning' below.

The x100 oil immersion objective is designed to be used with only synthetic microscope immersion oil. **Natural immersion oils like Cedarwood oil must be avoided because they dry to a crust on the surface of the lenses that can damage the lens surface** when cleaning off. A synthetic immersion oil with the DIN/ISO standard refractive index of 1.518 is generally recommended. Alternative immersion media such as water or glycerol may be used but if using such media it is important that any trace of a previously used medium be cleaned off the lens (and specimen coverslip) first. In general if you choose to change over to a different immersion medium any trace of the previously used used medium must be cleaned off the lens (and sample) before switching.

Cleaning

The objectives should not require routine cleaning other than removing oil or other immersion medium from the front lens of the x100 oil objective. However if the exposed lenses become soiled then the following guidance may be helpful.

If lenses are soiled by simple falling solid debris like dry dust, pollen etc. then an air duster may be used to blow these fragments off the surface of the lens. If this does not work then consult a specialist microscope technician for advice. **Wiping a lens that has dry debris on it (even with a special lens paper wipe) runs the risk of scratching the lens or its coating, so this type of 'wipe-cleaning' must be avoided in such circumstances**. A small amount of residual dry dust contamination will not usually need cleaning because it is unlikely to cause significant deterioration of the image. Over-cleaning or overly aggressive cleaning may do more harm in the long run.

If the front lens gets contaminated by immersion oil or grease (e.g. from a finger print) or other pure fluid then the lens may be cleaned using special lens paper with or without simple breath condensation or pure isopropyl alcohol. **Do not use domestic tissues or other cloths (because these may cause scratches to the lens or its coating and/or leave dust and debris behind). Do not use more aggressive solvents like acetone or xylene because these may damage the cements** used to keep the lenses in place.

For removing such fluid contamination first wipe off excess fluid with a dry lens paper. Then make a few more passes with a clean lens paper using either your breath condensation as applied moisture to the surface of the lens or put a drop of pure (99.9%) isopropyl alcohol onto the lens paper (not the surface of the lens) prior to wiping.

Always clean the oil (or other immersion medium) off the surface of the x100 oil lens after each imaging session and do not leave oil on the lens overnight when not in use.

When not in use, keep your objectives in their protective cases.

These lenses do not have any user-serviceable parts inside. Do not dismantle them.

As described in a previous section, if you are using an oiled condenser ensure that all excess oil is wiped off the top surface lens of the condenser after removing the slide at the end of your imaging session and remove oil from the surfaces of the slide as well. An ordinary household paper towel or tissue may be used for that purpose.

A tip to prevent any plastic dust of other debris from falling into the back of any objective lens is to use a clean plain round glass coverslip (17 to 18 mm diameter) in a filter slider in the lowest filter slot in the filter block (see figure 4). This ‘plain glass filter’ will act as a physical barrier preventing any plastic dust or debris from falling into the back of the objective as you manipulate the scope and insert and remove various ocular attachments and higher filters. From time to time you can remove this lower plain glass filter and clean it from any fallen debris. This is much preferable to cleaning the objective lens and in most cases it will not be significantly detrimental to image quality.

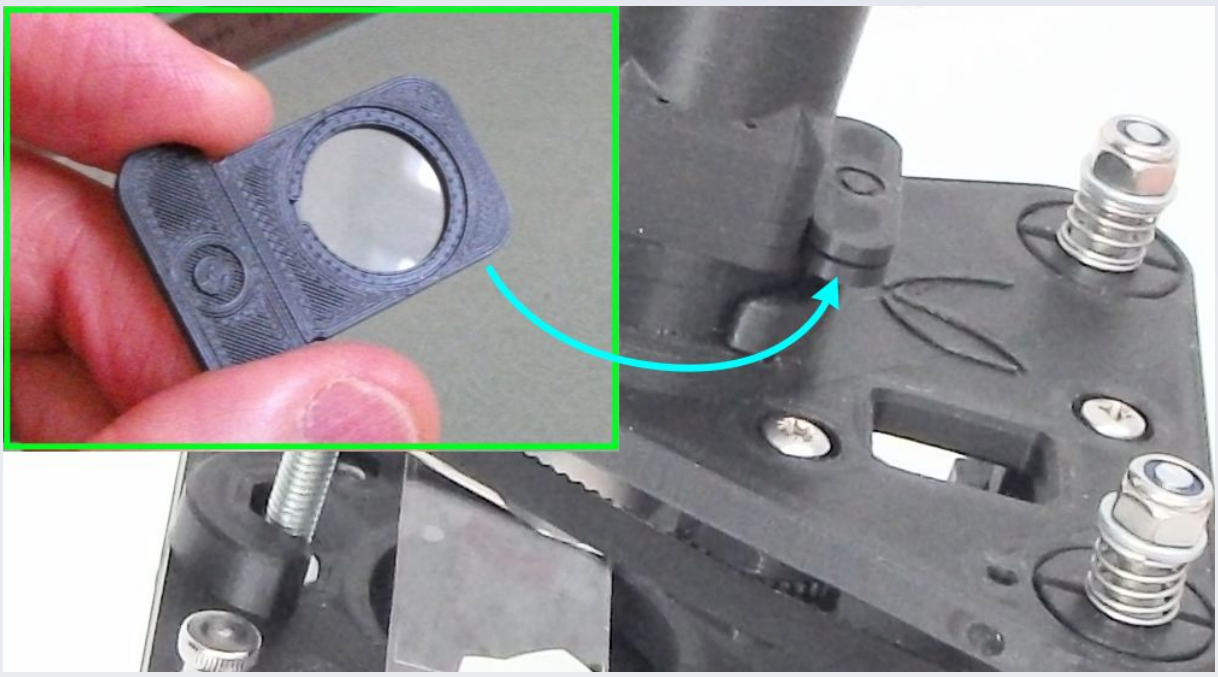

Figure 4. Protect your objectives from falling debris inside the optical tube by making a plain glass filter with a plain 18 mm diameter coverslip in a PUMA filter slider-holder as shown (inset) and keep this ‘filter’ in the lowest filter slot of your microscope.

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