UV transmission microscopy – optical imaging without glass

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Ultraviolet (UV) transmission microscopy is a technique which has been around for over 100 years. Originally developed in the pursuit of resolution, it has become less used over time due to the technical complexities involved and the rise of other methods. The aim of this work was to take an existing optical microscope (an Olympus BHB) and convert it to be able to image in the UV, down to and potentially even below 300 nm, to enable the imaging of sunscreen ingredients in topical emulsions. Every aspect of the optical train was assessed and changed to enable UV to be transmitted and imaged. Where required optical transmission spectroscopy was used to understand the behaviour of existing and new components. After conversion it was able to successfully image in the UV at 313 nm and 365 nm and to discriminate between different types of sunscreens based on their optical properties. While presenting a number of technical challenges to the researcher, it has been possible to convert a standard optical microscope into one capable of performing UV transmission imaging. The ability to directly image sunscreen components based on their optical properties in the UV region demonstrates the usefulness of the technique and shows it still has a place in modern microscopy.

Introduction

Ultraviolet (UV) transmission microscopy is a technique which has been around for over a hundred years. Kohler (1904a, 1904b) developed a microscope which took objectives from Zeiss which were corrected for the 275 nm Cadmium emission line, and demonstrated approximately twice the resolution compared with a similar visual light -based instrument. The technique itself presents a number of challenges as discussed by Taylor (1953). One complication with the use of short wavelength light in the UV is that glass becomes opaque. It can therefore not be used for optical elements in the microscope, and materials which are UV transparent such as quartz and calcium are required, or mirror -based optics can also be used. Combined with the need for complex and expensive optics, dangerous light sources, the inability to directly see the UV light by eye (and the need to protect the eyes of the operator of course), and the development of other techniques to improve resolution, the method of UV transmission microscopy gradually fell out of favour and became less common. It is still possible to buy commercially made UV transmission microscopes today, but they are hugely expensive and often need to be custom made.

Figure 1. Comparison of the original focus tube mechanism (left) with the custom made UV fused silica based focus tube (right) for the Zeiss HBO 50W light source.

In 2020 I decided that it was time to learn a new skill, and given a background in photographic imaging during almost 20 years of dermatology research, optical microscopy was chosen to build on that imaging experience. I decided to buy a microscope that was in need of repair and learn about the technique while fixing it. An Olympus BHB was sourced which was in need of repair, however it was simple to work on and mechanically was in good condition. It also came with a trinocular head, as the ability to use it for photography was an important consideration. Given a background in UV photography as part of a wider research interest in sunscreen development and use, I began to wonder whether it would be possible to modify the Olympus BHB to photograph in the UV down to around 300 nm to help with imaging of the emulsion structure within topical sunscreen products.

This article covers the key aspects of the conversion process, what needed to be considered and how the Olympus BHB was modified to be usable at such short wavelengths. It will discuss some of the interesting and unusual microscopy equipment which was used during the build, and will also show some initial sample images captured with it in the UV region.

Materials and Methods

Transmission spectra of the lens elements and filters, and measurement of the irradiance spectra of the light source involved an Ocean Insight FX spectrometer using approaches outlined previously by Crowther (2020). IVisible and UV images in Figure 10 were captured using a monochrome converted Nikon d800 camera using the custom adapted Olympus BHB described in the article.

The microscope build process

There were a number of considerations when starting this UV transmission conversion. Cost, convenience of use, safety of the user and reliability were all things which needed to be factored in to the build. Different components of the microscope needed to be modified and changed, as if even one piece of the optical train blocked the UV, then it would not be possible to use it for imaging. The following sections will take these individual aspects of the microscope and outline how they were adapted for the transformation to a UV transmission setup.

Light source

The light source had to provide strong emission in the UV region, while ideally minimising visible and Infrared (IR) light (to help with regards to filtering needed for photography). Light emitting diode (LED) light sources are becoming more and more

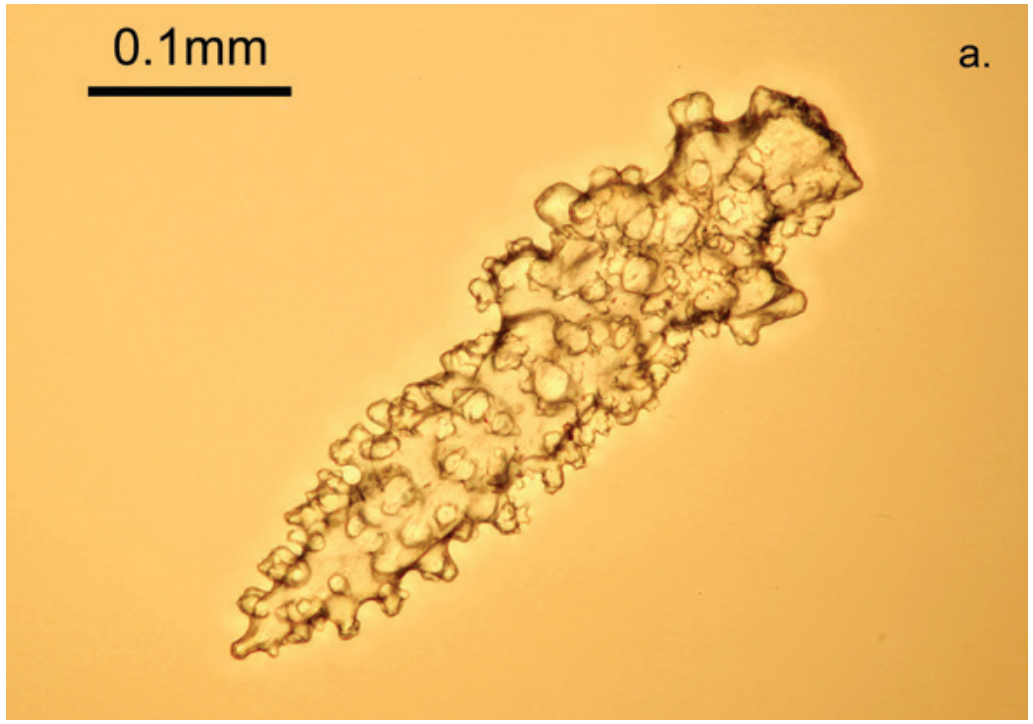

Figure 2. Sea fan spicule imaged in visible light with a 20x Olympus Splan objective using a Canon 5DS R camera and a) the standard Olympus Abbe condenser and b) the UV fused silica half ball lens as a condenser.

popular, and are readily available in the UV at 365 nm, however at shorter wavelengths, their light output drops rapidly and cost is high. These were therefore discounted for this work. Mercury Xenon lamps are often used for microscopy to provide a source of UV for fluorescence imaging, and units are available from many manufacturers. A second hand Zeiss 50W HBO lamp was sourced as the start point for the work. This is compact, the bulbs are readily available, and it has a basic focusing setup built into the lamp housing. The focusing setup needed modifying as the original lens was glass, and while this transmitted enough light for use at 365 nm, it was no good at 313 nm. It was therefore replaced with a 20 mm focal length fused silica aspheric condenser lens which was fitted inside a custom housing, Figure 1. This fused silica lens gave good transmission down to below 300 nm and allowed for focusing of the lamp. The other end of the custom tube was also threaded to allow for the fitting of a fused silica diffuser if needed. The lamp was fitted to the Olympus BHB microscope via a custom adapter. It should be noted that Olympus mercury xenon lamps are available for use with the BHB. These are typically 100W (requiring larger lamps) and the control box is much larger than the Zeiss one. As size was an important factor here, the Zeiss one was chosen for this work.

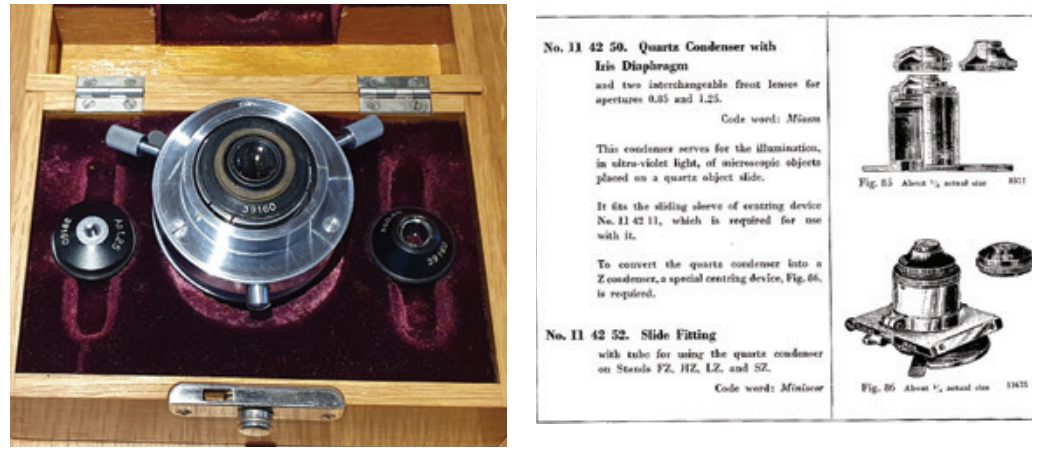

Figure 3. Zeiss quartz condenser, the condenser components (left) and an excerpt from the 1934 Zeiss Microscopes catalogue showing the condenser (right).

Internal microscope optics

The Olympus BHB has inside a number of glass lens elements and a mirror, before the light from the light source can reach the condenser. The field iris lens and auxiliary lens (which can be installed or removed depending on objective magnification) glass lenses were replaced with UV fused silica lenses to allow the shorter wavelength UV to pass. These were simple lenses – biconvex and planoconvex respectively – and were easy to swap out for UV fused silica ones. There is also a High – Low magnification selector on the microscope. In 'Low' magnification setting, no lens is present in the light beam, while in 'High' magnification, a lens is moved into the optical path, further focusing the light source. This lens was an unusual size, and was an optical doublet, making replacement with a UV transparent lens difficult. As the light source being used had a focusing lens on it, I therefore decided not to try and replace this lens, and to leave the selector in the 'Low' setting for all UV work.

There was also a mirror inside the body of the microscope. On testing, this showed to have good reflection properties down to 250 nm and so was not changed.

Condenser

In the initial work, I wanted to be able to do simple bright field imaging in the UV, so a suitable bright field condenser would be required. The standard Olympus Abbe condenser for the BHB was actually suitable for use at 365 nm, but being glass, was essentially opaque as the wavelength dropped below 320 nm.

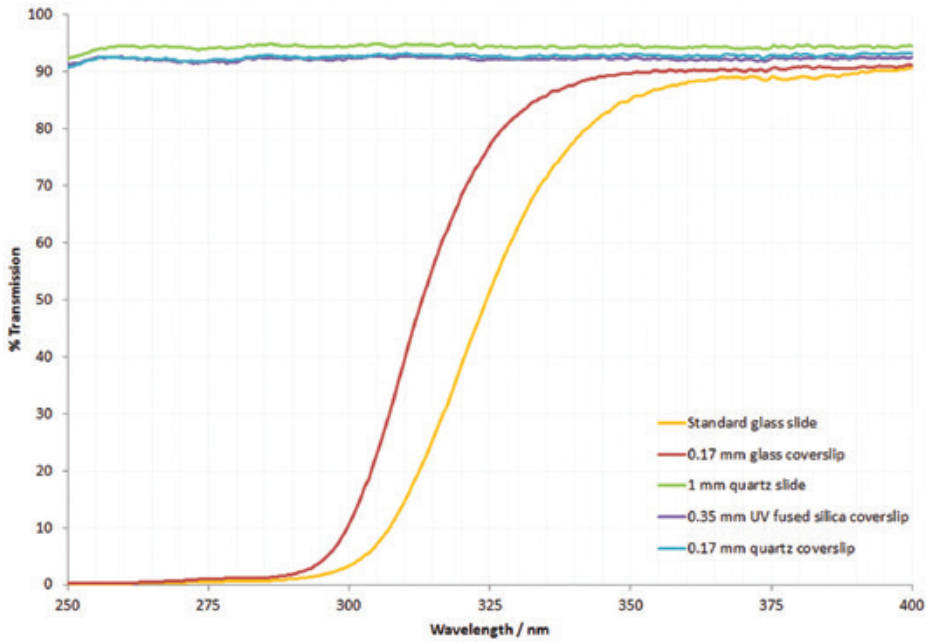

Figure 4. Transmission in the UV of standard glass slides and coverslips, and quartz and fused silica slides and coverslips.

For the first attempt to build a dedicated UV condenser, I bought a simple 8mm diameter half ball UV fused silica lens, and mounted this in an Olympus Abbe condenser mount in place of the original glass lens. Despite being a very different lens shape, I was able to use it to produce images, although with slightly lower contrast than the original Olympus Abbe condenser, Figure 2.

I knew that the homemade condenser was only going to be a temporary solution, and I was fortunate enough to find a Zeiss Quartz condenser for sale in the US. The exact age of it is unclear, but descriptions of it appear in a number of Zeiss catalogues from 1913 to 1934, which points to it being about 100 years old. It is shown in Figure 3 and consists of various quartz elements which can be assembled to produce a condenser suitable for objectives with a range of numerical apertures. Interestingly, it does not say 'quartz' anywhere on it, although transmission testing did indeed show that it was quartz and not glass. Even more fortunately, the diameter of the mount was exactly the same as the one on the Olympus BHB, so it could be used without further modification.

Slides and coverslips

As with the objectives and other optical components, the slides and coverslips could not be made of glass, as even though coverslips are thin, they block short wavelength UV light, Figure 4. Also, although not shown here, glass slides and coverslips tend to fluoresce under UV light. As such, quartz and UV fused silica slides and coverslips were sourced. Some of the objectives required 0.35 mm thick coverslips, and these had to be custom made. Needless to say, given their cost, quartz and UV fused silica coverslips and slides are not disposable items and great care needs to be taken when cleaning and handling them.

Objectives

A wide range of UV transparent objectives have been developed over the years. These are either based on quartz, a mix of quartz or fused silica with calcium fluoride, or mirrors, either with or without refractive elements. With hindsight the choice of the Olympus BHB as a the based model for this build was fortuitous, in that it is a 160 mm tube length microscope, and a number of old optical components are available for 160 mm tube length

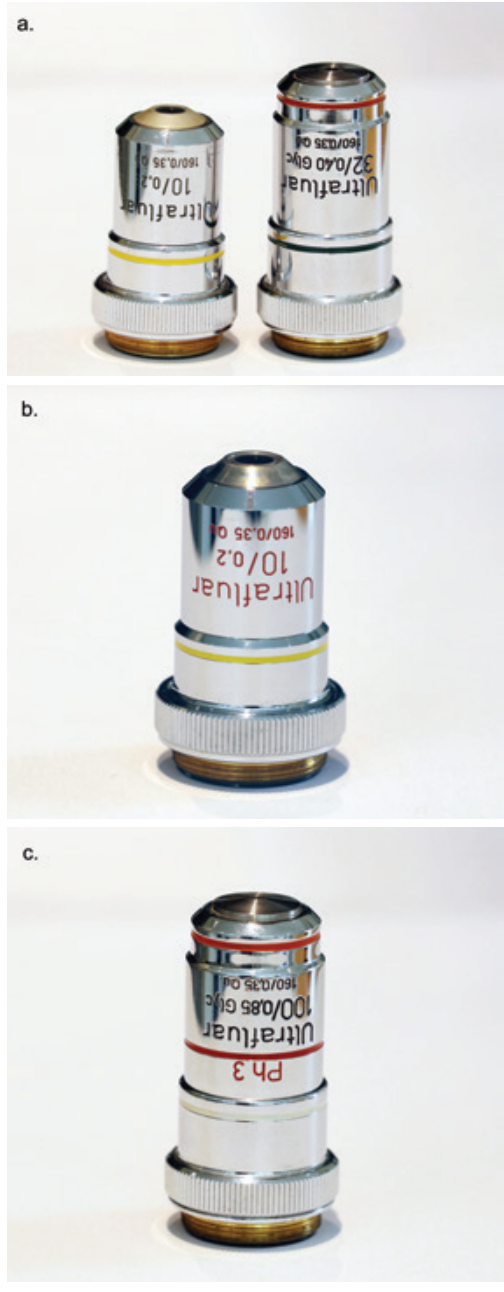

Figure 5. Zeiss Ultrafluars for 160 mm tube length microscopes, a) standard objectives in 10x and 32x, b) polarising objective in 10x and c) phase contrast 100x objective.

systems. After looking at a range of objectives, including reflective and refractive ones from Leitz and Lomo, Zeiss Ultrafluar objectives were chosen as the ones to use. These contain quartz and calcium fluorite lens elements, and are available in 10x, 32x and 100x magnifications, with some also being available as ones suitable for polarised microscopy (as denoted by the red text) and also for phase contrast, as shown in Figure 5. They have good transmission down to around 220 nm depending on the specific objective magnification and require very little refocusing when going from visible to UV. The 'standard' 160 mm tube length Ultrafluars do come up for sale relatively frequently, although the polarising and phase contrast ones are much rarer. This is another good reason to use a 160 mm tube length microscope as the finite tube length Ultrafluars are much more common on the second hand market than the infinite tube length ones. Even second hand they command quite high prices, and cheaper options for UV imaging include Lomo UV objectives and reflecting objectives, such as those sold by Beck or Ealing, which also appear regularly on the second hand market.

Trinocular head

The trinocular head proved to be one of the most challenging parts to modify. Inside the head there are two reasonably thick optical components made of glass. At the base of the head there is a thick window. As far as I can tell this is a window and not a lens, and I treated it as such by buying a UV fused silica disk in the correct size to act as a replacement. The more complicated part to deal was the moveable beam splitter. This is slid to change between all the light going to the eyepieces, and the light being split between the eyepieces and the port for the camera. A custom made beam splitter / prism would have been too expensive, so instead I had the existing one cut in two. One half was kept as standard, for diverting the light to the eyepieces. The other side was replaced with a simple fused silica block with the same thickness as the glass part that was removed. These two pieces were then glued together and mounted back in the slider in place of the original beam splitter.

Safety of the user was a primary concern for this build, and with the eyepieces still in use there is the potential risk of exposure to significant levels of UV reaching the eyes. In addition to other safety measures which will be discussed further below,

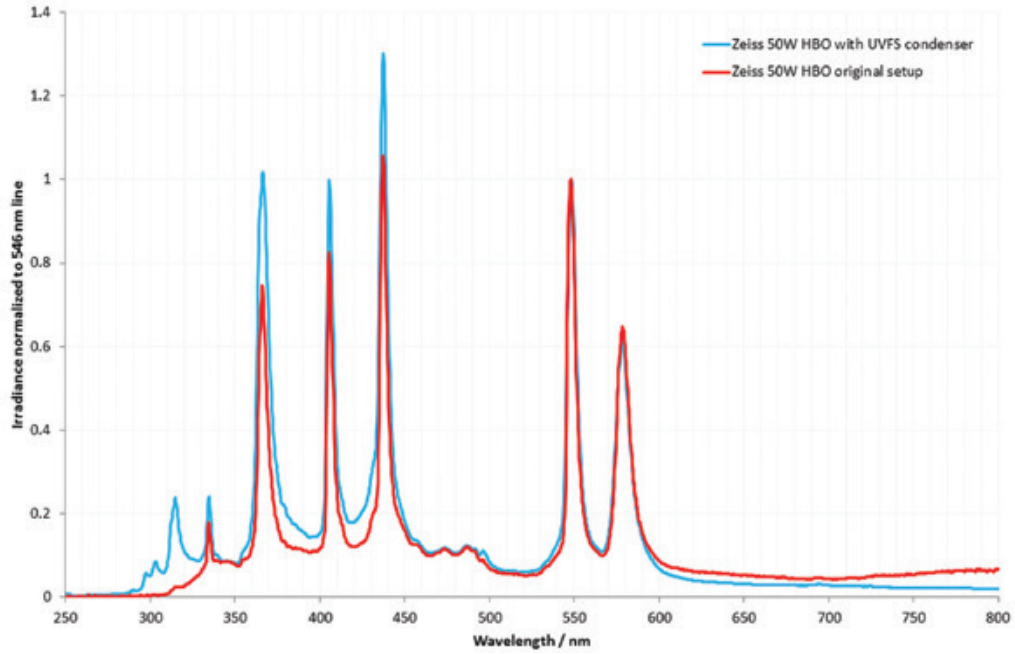

Figure 6. Zeiss HBO irradiance spectra normalised to the mercury 546 nm line, with the original setup and with the fused silica condenser lens installed.

deep yellow camera filters were installed in the eyepieces for the microscope. These have strong blocking in the UV (>99.99% blocking below 400 nm).

Photo eyepiece

The Olympus BHB relies on the use of a photo eyepiece to create an image which is then projected directly on the camera sensor without the need for further optics. The normal Olympus photo eyepieces are excellent for visible light use, but as they are made of glass they were not suitable for short wavelength UV imaging. A range of quartz eyepieces which have great UV transmission down to and below 250 nm, have been produced by different manufacturers, although most are difficult to find on the second hand market. However, the ones made by Lomo do come up for sale more frequently, and are the same diameter as the Olympus ones so can be fitted to the trinocular head without further modification. Some of these are corrected for specific Lomo UV objectives, while others are un-corrected. If possible, it is worth testing a number of these to determine the best image with the specific objective lenses being used.

Filters for imaging

The Zeiss HBO mercury xenon light source has strong emission bands in the UV at 313 nm and 365 nm as well as a strong line in the visible spectrum at 546 nm, Figure 6. The UV line intensities are improved relative to the 546 nm line by the use of the UV fused silica condenser lens as expected. The visible light line can be imaged by placing a Zeiss Mercury (Hg) 546 filter on top of the field iris lens, which eliminates the UV light. For imaging at 313 nm and 365 nm, 10 nm bandpass filters are placed on top of the photo eyepiece, and the other filters are removed from the front field iris lens. Transmission spectra of the filters used are given in Figure 7. As a result of the design for imaging in the UV, the full spectral output of the light source illuminates the sample, and passes through the objective and photo eyepiece before being filtered. Filtering above the sample and photo eyepiece is recommended over filtering below the sample for the following reasons. Firstly, if the UV causes fluorescence in the sample, with the filter being the final stage before the camera, this fluorescence will be removed. If the UV bandpass filter is below the sample, any fluorescence will be imaged along with the transmitted UV. Secondly, larger filters would be needed if they were placed on the field iris lens, and as these are expensive items, this helps reduce overall cost.

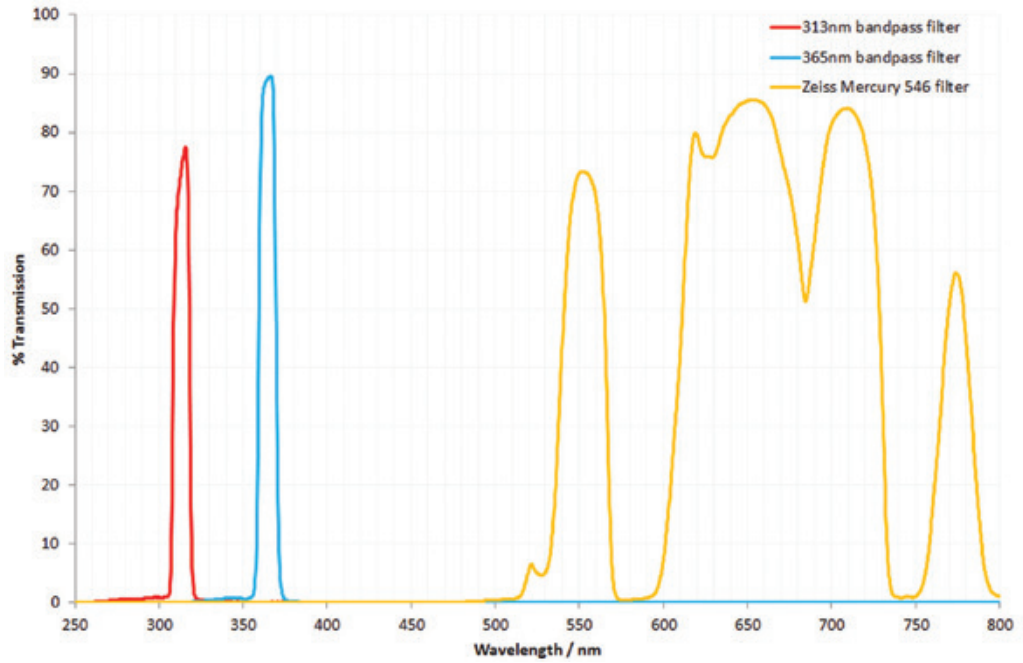

Figure 7. Transmission spectra of the 313 nm, 365 nm and Zeiss mercury 546 nm filters.

It should be noted that at both 313 nm and 365 nm, two bandpass filters were used for each wavelength and they were stacked on top of each other. While blocking of the filters was advertised as providing <0.01% transmission in the out-of-band regions, the reduced camera sensitivity in the UV combined with the lower intensity of the light source at 313 nm meant that degree of blocking was not sufficient to produce a clean 313 nm image. Stacking two 313 nm filters together hugely improved blocking of the unwanted wavelengths, resulting in a clean image. Two filters were also used at 365 nm to keep the thickness the same as for the 313 nm filter stack, and reduce the need for refocusing when moving between the two UV wavelengths.

Camera

Image capture is done with a modified high street Single Lens Reflex (SLR) camera. This allows the images to be captured without the need for a computer to run the camera. Also live view can be used to see the images in the visible spectrum, and in UV at 313 nm and 365 nm enabling any focussing to be done. Although an SLR camera, it has been modified to allow it to see the UV. The camera has had the internal UV and IR blocking filters removed, making it sensitive to the UV and IR as well as visible light. However, it has been further modified, having the Bayer filter and microlens array removed to reveal a bare sensor which captures monochrome images. A fused silica coverglass is then put over the sensor to protect it. This has the effect of drastically improving UV sensitivity, as the Bayer filter dyes absorb a significant amount of any UV before it can reach the sensor, Crowther (2019). This conversion enables visible and UV to be imaged, even at 313 nm, using the same camera, although the ISO setting and exposure time on the camera do need to be changed for each wavelength.

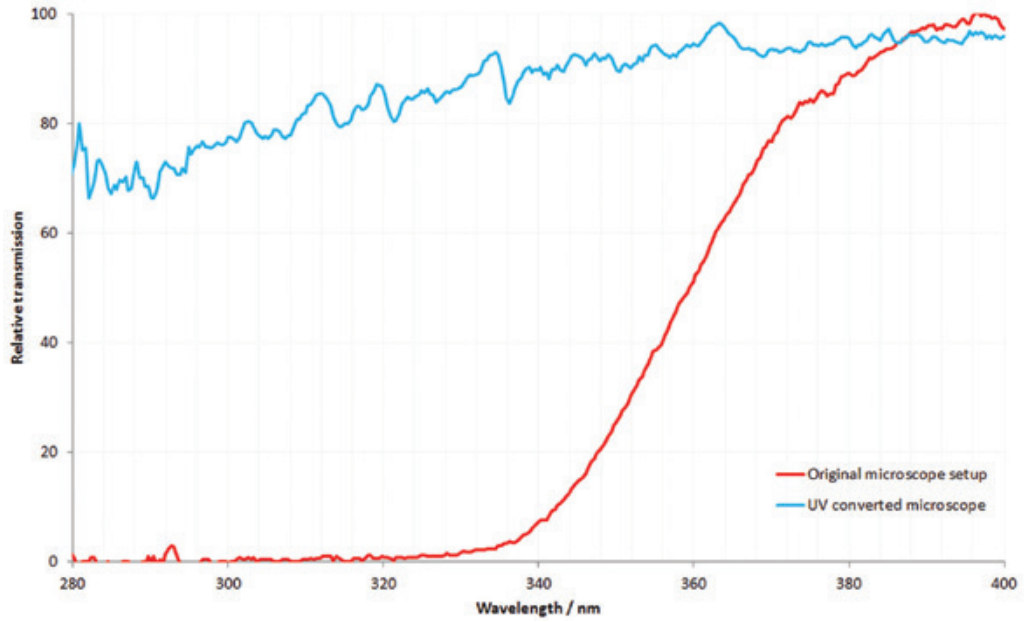

Figure 8. Light transmission through an unmodified and the UV converted microscope in the UV region.

Depending on the objective, images can be collected individually or as a stack, although with the samples the author is currently imaging, single images are preferred as the sunscreen samples are quite mobile, producing artefacts in stacks.

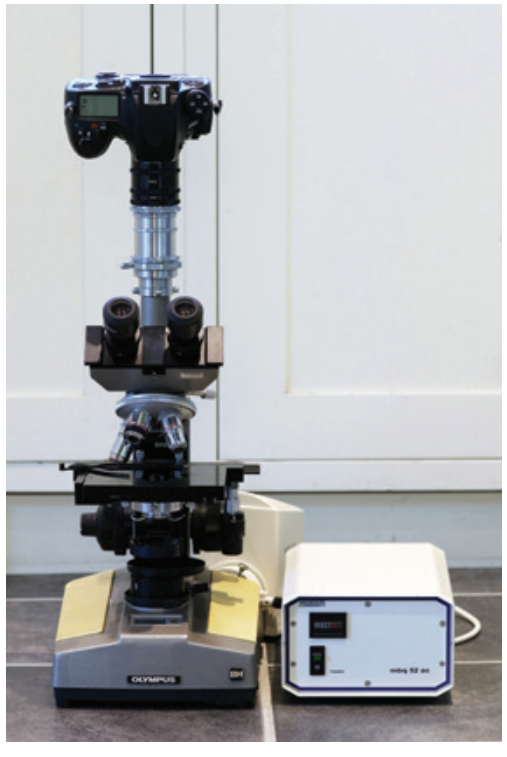

Figure 9. The overall appearance of the device.

Safety

UV light is very dangerous to the skin and eyes, especially the shorter wavelengths around 300 nm. Given that highly focused UV is being used to illuminate the sample, a number of safety features are employed by the author to prevent the risk of eye and skin exposure;

- UV safety goggles which wrap around the side of the head are worn at all times when using the microscope.
- Unless UV imaging is being done, a Zeiss 546 Mercury filter, a UV/IR cut filter, and a 6 stop neutral density filter are placed on top of the field iris. This means that only the light from the visible region is reaching the sample.
- Yellow filters are placed below the eyepieces in the binocular head. These have a transmission of <0.01% below 400 nm.
- Live view is used on the camera to check the focus for photographic imaging.

In addition, the author wears long-sleeved clothing when using the microscope to reduce the chance of any reflected light reaching the skin when the filters which block the UV are removed from the field iris. The risk of damage to the eyes especially cannot be overstated with this type of device, and anyone attempting this type of work should always keep that in mind. Having access to a spectrometer which can measure irradiance spectra is strongly recommended. It would be interesting to know whether the early pioneers of UV transmission microscopy were aware of the risks associated with the use of the light sources they were using and how many suffered eye damage as a result of their work.

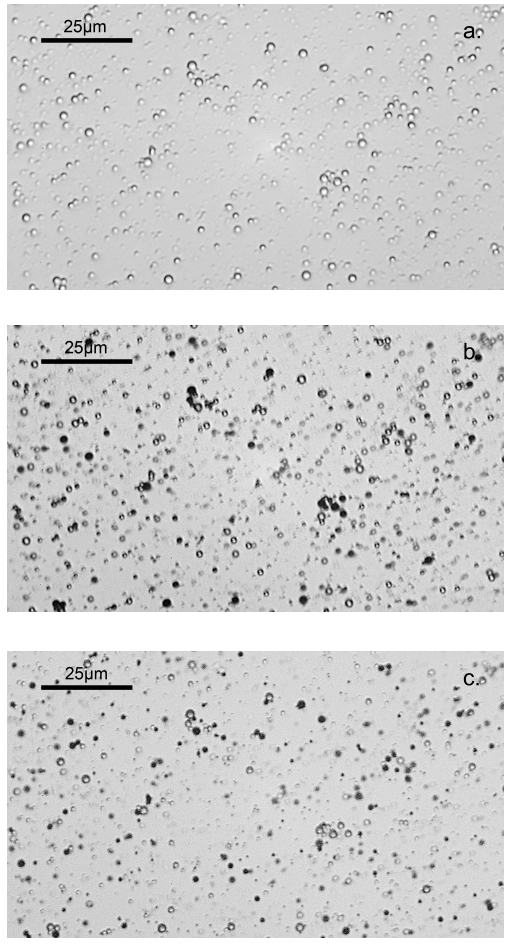

Figure 10. Images of the mixed sunscreen dispersion in, a) the visible light region using the Zeiss Mercury 546 filter, b) the UVA at 365 nm and c) the UVB at 313 nm.

Effect of conversion on optical transmission

The overall effect on UV transmission of the microscope conversion is easiest to see when looking at the relative transmission of light through the converted microscope compared with one which has not been converted, Figure 8. Before conversion imaging at 365 nm would have been possible with the Olympus BHB, but with the wavelength reduced to 313 nm no light would be able to pass through the microscope.The conversion has resulted in good transmission down to 280 nm and it is likely based on the components used, that this would still be usable at 250 nm, although with some loss of light.

Final device and initial UV transmission images

The final layout of the entire device is shown in Figure 9. Did the build meet the initial design criteria? It is certainly compact, being no larger than the original Olympus BHB. While the light source is an add-on, requiring a power supply, this does not significantly add to the bench space needed for the device. The camera is stand-alone, so there is no need for a computer. Unlike older designs of UV transmission microscopes, no complex monochromator was needed, as for example described in Foster and Thiel (1948) or Land et al. (1949), which helped keep the size down. Instead of the monochromator, dichroic filters were used to filter the light before it reached the camera.

All the objective lenses were bought second hand, as was the quartz condenser and photoeyepieces. However, the fused silica optical lenses and windows and the UV filters needed to be bought new, and some custom machining work was required to mount the light source which added to the cost. Overall, the build price for a basic setup with a couple of objectives came to a few thousand GBP (not including the camera which the author already had), which is certainly much less than the price of a new UV transmission microscope. The main driver for the complexity of this build was the

requirement to operate in the short wavelength UV region below 320 nm which meant moving away from glass optics to quartz and fused silica.

As a proof of concept for the device, imaging of sunscreen products has been done. The sample was prepared by dispersing a UVA active sunscreen and a UVB active sunscreen in water, and then mixing these dispersions together. Both sunscreens were oil in water emulsions with the UV absorbing ingredients in the oil phase. This results in some oil droplets containing UVA absorbing actives and other oil droplets containing UVB absorbing actives. Images were taken in visible light (546 nm), in the UVA region (365 nm) and in UVB (313 nm) using a 32x Zeiss Ultrafluar objective, Figure 10.

The visible light image (Figure 10a) shows the expected oil droplets in a continuous water phase. The oil droplets show no strong absorbance in the visible light region and all appear clear and similar. In the UVA image (Figure 10b) some of the oil droplets now appear dark, while others remain clear. The dark droplets are the ones containing the UVA containing active. When imaged in the UVB range (Figure 10c) the droplets which were clear in the UVA image now appear dark, and those which were dark now appear clear. Other than sharpening of the images, no other processing has been done and no complex sample preparation was required. There has been some movement of oil droplets between the images due to the low viscosity of the sample. Brownian motion keeps the droplets moving in the water phase, and in the current setup the camera needs to be removed and then replaced when changing the UV filters. These are initial images captured using the microscope and further work is now planned to optimise and improve the imaging process.

Conclusions

UV transmission microscopy certainly presents a number of challenges when compared with normal visible light transmission microscopy, from the absorption of the light by normal glass, to the light sources required, and the sensitivity of the camera. In addition the safety of the operator needs to be carefully considered and controlled. However, revisiting this historic technique has enabled direct visualisation of sunscreen components in different regions of the UV spectrum without the need for any complicated sample preparation, demonstrating that there is still a place for this approach in the research world today.

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I have worked for 18 years in the cosmetics and pharmaceutical industries, in fields ranging from new product research with novel materials through to developing and running clinical studies to provide claims support data for product launches. Even when in a corporate environment I worked across multiple project areas at any one time to translate the needs of the individual teams into executable study designs and plans. Research areas worked on include moisturisers, cleansers, and hair removal methods. In 2012 I set up JMC Scientific Consulting Ltd and now work with clients across the globe.

Photography has always been a passion of mine. A principle area of focus for my research is UV imaging and how it can be used to visualise skin and sunscreens. UV imaging is extremely challenging, presenting a unique set of complexities, and my research often requires me to build equipment to help with my work where nothing suitable is commercially available.

I have a very hands on approach to science and am experienced with the operation and use of a wide range of skin testing equipment including Confocal In vivo Raman Spectroscopy (for hydration profiles and ingredient penetration), photographic imaging techniques, skin grading, tape stripping and SEM and clinical test design.

After building my expertise in the skin methods field I also became more involved with teaching the science of skin measurement, to audiences ranging from Dermatologists to Journalists and Marketing groups. I am also strongly linked with Academic research, having overseen a number of projects with different universities. I have authored over 40 papers and book chapters, with an emphasis on skin measurement and imaging.

I graduated from Durham University with a BSc in Chemistry in 1994 before doing a PhD in Surface Modification and Analysis which I completed in 1997. Research was such a passion of mine that I stayed on for an extra 3 years as a Post Doctoral research assistant. During this time I developed a strong analytical chemistry background with a wide variety of surface analytical techniques (XPS, Auger, ToF SIMS, Raman, ATR-IR, SEM and TEM, AFM) for materials analysis, along with cold plasma treatment of materials for the manufacture of metal surfaces and ultra-low energy materials.

It is the cross discipline background I have from working at the boundaries between chemistry, physics and engineering, which I have now applied to my assessment of skin. I apply a strongly analytical approach to my work and am a scientist through and through, driven to question and understand how the world works.