A Look at UV-Curing Mounting Media and Notes on Index of Refraction

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Introduction

Ultraviolet-curing adhesives have been used as mounting media for about 35 years. The first in widespread use was Norland Optical Adhesive 61 (Figure 1A), used from the mid 1980s to the present. A 1997 *Micscape* article by Alan Brinkworth and Maurice Smith describes the use of Loctite Glass Bond adhesive as a mounting medium. In the years since then, the once exotic UV-curing adhesives have become commonplace and have proliferated into a variety of products. One development is the all-in-one package containing both glue and a UV-emitting LED curing lamp (Figure 1B). On another front, it is a boon to microscope users that ornately decorated fingernails are popular among the young and trendy; products intended for nail decoration can provide both mounting medium (Figure 1C) and slide curing oven.

I've used Norland 61 for a long time. It works well, but has a couple of inconveniences: it has a short shelf life, and it is expensive. A few years ago, when I noticed the glue pen with built-in UV light, I made some test slides to see how other UV-curing products compared with Norland 61.

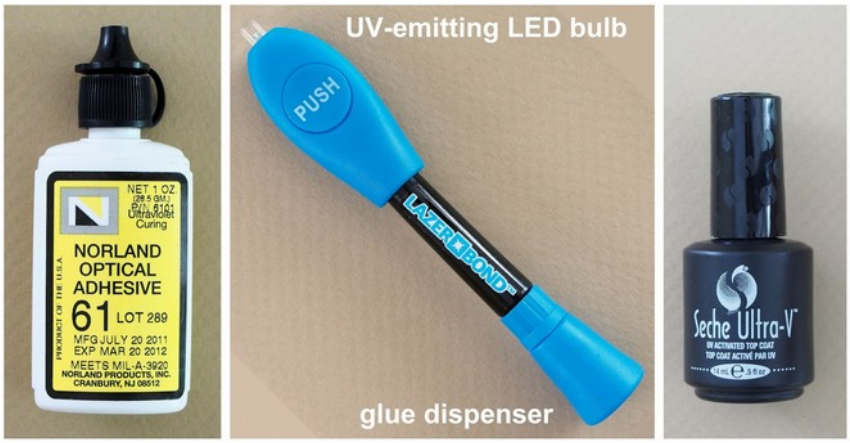


Figure 1. *The UV-curing products considered in this article.* ***A.*** *Norland Optical Adhesive 61.* ***B.*** *"Lazer Bond" brand adhesive, a glue pen with a built-in UV-emitting LED (not a laser).* ***C.*** *Seche Ultra-V UV-curing nail polish top coat.*

Safety Precaution. UV-curing lamps use near-visible light at 365 nm wavelength. This wavelength is not overly dangerous, but some lamps might also emit the more dangerous shorter wavelengths blamed for a number of serious eye and skin problems. You should protect your eyes when curing microscope slides with UV light; wearing UV-blocking sunglasses is an easy solution.

Adult supervision is required for the methods described in this article!

Example 1: Dry Mount Cat's Claw

Figure 2. *A dry mount using Lazer Bond UV-curing adhesive to attach slide components.*

Figure 2 is a dry mount with the specimen in air. The specimen, spacer ring, and cover slip were attached with UV-curing adhesive. The slide is in the spirit of Victorian microscopists who were fond of birefringent objects "for the polariscope" such as horse hair, rhinoceros horn or, as seen here, nails and claws. In Figure 2, the specimen and spacer ring are illuminated by transmitted cross polarized light; reflected light was added for the slide label. Colors are due to **form birefringence** in the specimen (bundles of parallel fibers), and **stress birefringence** in the plastic spacer; both of these differ in origin from the more common **intrinsic birefringence** seen in mineral crystals.

Components. Cats periodically grow new claw sheaths and shed the old ones. I found this example near my cat's scratching post. The spacer ring is a 2mm thick translucent plastic washer from the disposable packaging of a stack of recordable DVDs. The pattern for the computer-printed Victorian style label is available in an earlier *Micscape* article.

Lessons Learned. In this example, the UV-curing adhesive is a glue to hold slide components together, not a medium to embed the specimen. This particular adhesive (Lazer Bond brand) sticks well to a variety of substances: glass, specimen, and plastic. Some optical glues intended for glass have poor adhesion to other materials; this property varies with the brand and must be determined by trial and error. The adhesive is clear in thin layers. It is isotropic, making it suitable for polarizing specimens. The permanent mount slide with three separate gluing steps was ready for use in less than 15 minutes – except for the fancy label; that took longer.

Why Use UV-Curing Adhesives?

UV-curing adhesives are plastics that harden by a change of state triggered by exposure to particular wavelengths of light. Unused adhesive should be stored in the dark and not exposed to much light before a slide is cured.

UV-curing adhesives have some characteristics that make them good slide adhesives and mounting media. They are safe to use if you protect your eyes from UV exposure. Lack of a solvent means they don't have strong chemical odors, don't give off many volatiles, and don't lose volume as they cure. UV-curing adhesives are inert, once cured. They do not chemically react with most specimens (pH neutral). They do not cause biological stains to fade. They do not crystallize or break down with age (35 year track record). They do not react with atmospheric oxygen, so it is not necessary to seal the edges of the cover slip. They harden quickly; when cured by lamp, permanent mounts are available in minutes, rather than the hours, days, or even weeks necessary for some solvent-based media to harden. These materials are not sold as "microscope slide mounting media"; even the old standby Norland 61 is described as a "military grade optical adhesive". Because many UV glues (other than Norland) are consumer products, they are easy to find and are relatively inexpensive.

The Importance Of Index Of Refraction: Optical Relief

The three UV-curing materials considered in this article have different **indexes of refraction** ("n" or "R.I."). These R.I.s determine the subject material for which a particular mounting medium is best suited (Figure 3). Our interest in index of refraction centers on the concept of **optical relief**, contrast caused by the difference in R.I. of specimen and mounting medium. A colorless low relief specimen with an R.I. close to the mounting medium is nearly invisible. In axial light, it is the magnitude of the difference, either higher or lower, that affects visibility. The classic low relief subject is cytoplasm (n=1.35) in water (n=1.33). Phase contrast and other contrast enhancement techniques were largely developed for cell biologists to overcome this situation. Moderate relief specimens are visible but can appear ghost-like with indistinct edges. High relief specimens (optical relief greater than about 0.12) stand out crisply. Large, thick shelled diatoms look good in Norland 61 (optical relief 0.13). Small thin shelled diatoms with very fine structure, including all the species on most diatom test plates, look better in a high index medium (optical relief about 0.3).

Figure 3. *Index of refraction of some mounting media and subjects. Items in red are considered in this article. Index of refraction values were tabulated from numerous sources, both online and in print.*

MOUNTING MEDIUM	Index of Refraction
air	1.0003
water	1.333
glycerin jelly	1.443
Karo (fructose corn syrup)	1.484 to1.486
Biological media: Euparal, Histoclear, Permount, Clearmount, etc	1.48 to 1.51
Loctite 3493	1.48
Nitrocellulose (nail polish)	1.50
homogeneous immersion oil and glass slides	1.515
Lazer Bond glue	1.52
Canada balsam (variable)	1.52 to 1.54
Norland 61	1.56
Caedax (no longer made)	1.57
Styrax	1.58
High index diatom media: Hyrax, Pleurax, Naphrax, Zrax	1.70 to 1.80
Realgar	2.4

SUBJECT MATERIAL	Index of Refraction
Biological	
cytoplasm	1.35
cell walls	1.42
cell membrane (variable)	1.46 to1.60
hair (average value)	1.55
Mineralogical	
Bio-silica (opal): sponge, diatom, radiolarian, ebridian, silicoflagellate, phytolith	1.434
Quartz	1.544, 1.553
Calcite: coccolith, foram, ostracod, some mollusc, echinoid, statolith	1.486, 1.640
Aragonite: some mollusc, including pteropods. some forams	1.530, 1.681, 1.685

You can go too far in the quest for optical relief. Very large differences in R.I. bring up the problem of **total internal reflection** which causes heavy black outlines and loss of data in the dark borders. An extreme example of high optical relief is an air bubble in the medium.

When might you not want, or not need, optical relief? In laser confocal microscopy, for example, a difference in R.I. is called an "index mismatch", and it lowers the resolution of the system. In visual microscopy, optical relief is not so important if contrast is provided by another means such as inherent color, or staining, or enhancement with phase or DIC. For classic brightfield microscopy, though, it is still a good idea to use a medium that provides optical relief for the subject. In order to do that, you need to know the medium's R.I.

A Method To Measure Mounting Medium Index Of Refraction

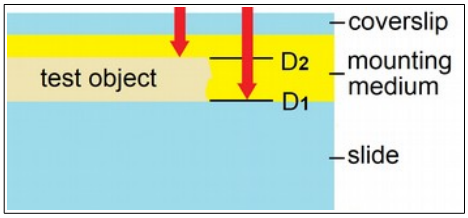


Figure 4. *This schematic cross section shows measurements to determine thickness of a test object, from which the R.I. of the mounting medium is calculated. The same measurement is made in air (for true thickness), in water (as a check), and in the mounting medium (for apparent thickness). Focus the D1 level through empty mounting medium, not through the test object.*

If an R.I. value is not provided by the manufacturer – and it probably won't be for consumer UV adhesives – you can calculate the value yourself. The no-cost method described here doesn't require additional equipment or index matching liquids. It uses the microscope's calibrated fine focus to measure the same Z-axis distance in air and in the medium. The relationship is:

(R.I. of Mounting Medium) = (True Thickness in air) / (Apparent Thickness in medium)

This equation *can* produce an exact value, but results depend upon subjective determination of best focus, and a difference of 1 µm makes a difference in the calculations. You should think of the calculated R.I. as approximate, unless you are *extremely* careful and can reliably repeat results. R.I. varies with wavelength (**dispersion)** and temperature. Standard R.I. is for the 589 nm sodium D-line (yellow) at 20C (68F). Although I have a 589 nm interference filter, I normally use a green filter in this calculation, giving an R.I. value for the mid range of visible light.

- **True Thickness.** A test object is attached to a slide. Ink dots on the slide top and object top are focusing targets. Add a temporary coverslip. With the object in **air**, measure thickness, which is D1-D2 in Figure 4.
- **Accuracy Check.** Add **water** under the coverslip; this serves as a temporary medium of known index. Measure thickness as in Figure 4 and calculate R.I. Results should be close to 1.333 (1.335 in green light).
- **Apparent Thickness.** Remove the coverslip and dry the components. Make a permanent mount with the **medium** you are evaluating. Measure thickness as in Figure 4. Calculate the medium's R.I.

Example Calculation For Lazer Bond Adhesive

Measurement of reference thickness (D1-D2 in Figure 4):

In air	111 µm
In water (as a check)	83 µm
In cured Lazer Bond	73 µm

Calculations:

R.I. of water	= 111 / 83 = 1.337 (check is close enough; correct value for green light is about 1.335)
R.I. of cured Lazer Bond	= 111 / 73 = 1.521

Mounting Media Considered In This Article

Prelude: Caedax Mounting Medium. This solvent-based "artificial Canada balsam" from Merck in Germany was the medium of choice for microfossils and marine sediments for many years. We will see one of my old Caedax slides later in this article. Beginning in 1979, various countries banned Caedax as part of a general ban on polychlorinated biphenyl (PCB). Several years passed before a good replacement for Caedax was found.

An Aside: If you are searching for properties of discontinued media such as the PCBs Caedax and Aroclor, an article by Paul A. Brown on curating slide collections has valuable information about mounting media of the past.

Norland Optical Adhesive 61. In the mid 1980s, UV-curing Norland 61 was found to be a good replacement for Caedax. It has nearly the same R.I., and is even easier and faster to use. Norland 61 now has a successful 35 year track record mounting subjects that would have been mounted in Caedax before 1979. An online discussion by a group of diatomists provided background information: Victor Porguen, an amateur diatomist from Los Angeles, was an early user of Norland 61 as a mounting medium. He brought it to the attention of the Ocean Drilling Program, where it became standard protocol for shipboard slide preparation. Norland's website provides data sheets with R.I. and other information. Norland 61's high viscosity can leave voids unfilled; warming before curing helps. Norland 61 has a stated shelf life of only 8 months from manufacture; low-volume users won't finish a bottle before the official expiration date. As a mounting medium – as opposed to a "military grade optical adhesive" – the expiration date is overly pessimistic. If the unused adhesive is stored in a cool, dark place, it will last. I have successfully used Norland 61 two years beyond its expiration.

Consumer UV-Curing Adhesives. The one tested here – **Lazer Bond** brand – is only one among many; most adhesive manufacturers have UV-curing adhesives in their product line. **Bondic** and **5 Second Fix** are two other all-in-one packages that include an LED curing lamp. Index of refraction probably varies between brands, and could be a major factor in choosing one over another. I measured the R.I. of Lazer Bond adhesive to be n=1.52. Lazer Bond handles much like Norland 61. It has high viscosity, which means warming before curing helps fill specimen voids. It does a good job adhering to a variety of materials, as demonstrated in Example 1.

UV-Curing Nail Polish. The Seche Ultra-V UV-activated nail polish turned out to be a surprise and a disappointment. The problem is that it is a composite medium. It does contain a UV-hardening agent but, like normal nail polish, it also contains nitrocellulose and some unpleasant solvents. It boils violently when heated and shrinks significantly when cured due to loss of solvents. It also makes your work room smell like a nail salon, which is not healthy. The nitrocellulose base has a published R.I. of 1.5. I made successful preparations by adding additional medium under the edge of the coverslip to replace the volume lost to solvent evaporation. I can not recommend the UV-curing nail polish as a general purpose mountant, but it has an advantage when you want an R.I. significantly lower than that of Norland 61.

Curing Slides

Slides cure in a few hours in sunlight, or in seconds to minutes with UV-emitting lamps. A curing oven is convenient if you mount many slides. The oven in Figure 5 is an inexpensive nail polish oven available new on eBay for about US$20.

Figure 5. *Curing UV adhesives.* ***A.*** *Curing one slide at a time with the LED built into the Lazer Bond glue pen. A UV filter from an old halogen desk lamp provides eye protection.* ***B.*** *Curing several slides at a time in a UV nail polish curing oven. A swing-down cardboard door on a tape hinge was added to block UV light while the oven is in use. Compact fluorescent bulbs emitting UV at 365 nm cure a tray of 8 slides in a minute.*

Example 2: *Discoaster* In Pliocene Deep-Sea Sediment

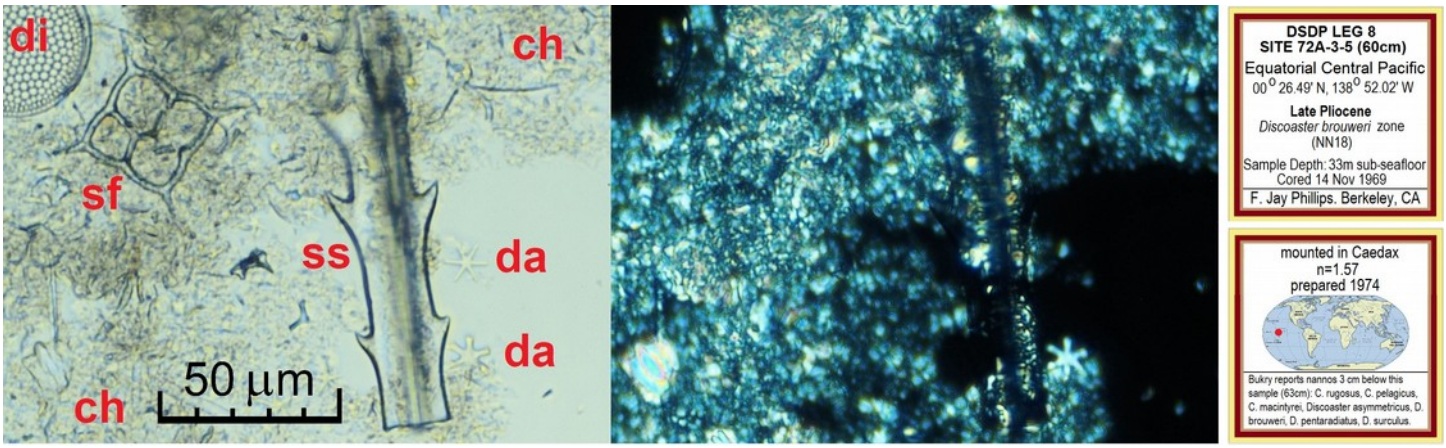


Figure 6. *A Deep Sea Drilling Project sample prepared in Caedax and imaged with a 20X objective in plane polarized and cross polarized light. Slide labels give location and preparation information. Key:* ***ch****=carbonate hash of fine-grained disarticulated shell fragments and coccoliths;* ***da****=Discoaster;* ***di****=diatom;* ***sf****=silicoflagellate;* ***ss****=sponge spicule.*

This Late Pliocene (about 2 million years old) sample from the equatorial central Pacific contains abundant calcareous and siliceous microfossils, including several species of the star-shaped calcareous genus *Discoaster*. Figure 6 is a good illustration of the effect of optical relief on subject visibility. The mounting medium index n=1.57 favors silica (relief 0.14) over calcite (relief 0.07). This results in siliceous components (labeled di, sf, and ss) standing out more boldly in the image than calcite components (labeled ch and da). The scientific report for the drilling cruse that collected this sample is available here; chapters 13, 14, and 15 discuss these fossils. For a guide to microfossils found in deep sea sediments, it is hard to beat the official shipboard manual for sample description, available here (large file; loads slowly).

This sample is close to the end of the line for *Discoaster*. The genus evolved in the Paleocene, but after a run of 60 million years, the group becomes extinct a few stratigraphic meters of section above this sample. In fact, the end of the Pliocene in tropical deep sea sediments is often defined as the extinction of *Discoaster*.

In Figure 6, one of the *Discoaster* specimens is dark in crossed polars, but the other is bright. This is due to the orientation of each specimen on the slide. Figure 7 shows a high resolution 3D view of a *Discoaster* as seen in the scanning electron microscope. Figure 8 explains the characteristic and distinctive "now-you-see-it, now-you-don't" structure of *Discoaster*.

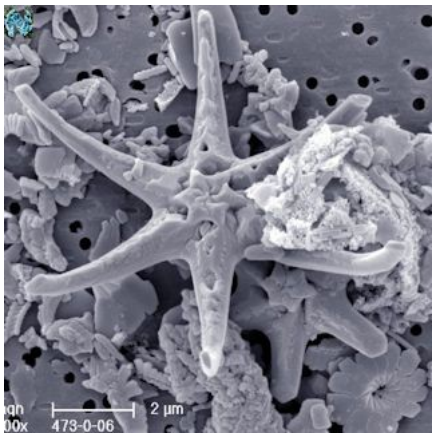
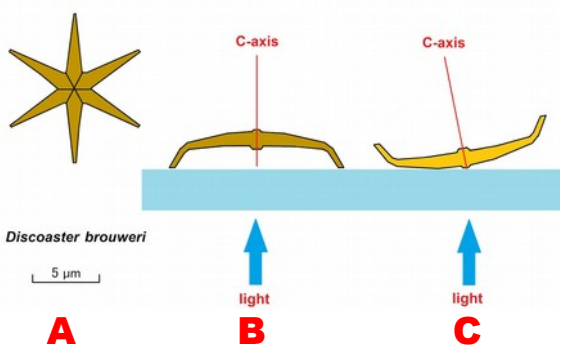


Figure 7. *Scanning electron micrograph of Discoaster brouweri. Photo by Jeremy R. Young, University College, London. From Nannotax3.*

Figure 8. *Seeing Stars. Visibility of Discoaster in cross polarized light is a matter of chance, depending upon the orientation in which it settles onto the slide.* ***A.*** *Plan view. Each arm is a calcite crystal with its mineralogical C-axis perpendicular to the arm (coming out of the page).* ***B.*** *A cross section of a Discoaster "sitting level" on the tips of its arms. The C-axis of each arm is aligned with the polarized light beam, making the specimen apparently isotropic and dark in cross polarized light.* ***C.*** *If the Discoaster lands the other way up, it might be propped at an angle so the C-axes of the arms are not parallel to the light beam; the specimen is now bright in cross polarized light. Discoaster sketches modified from Jeremy R. Young in Nannotax3.*



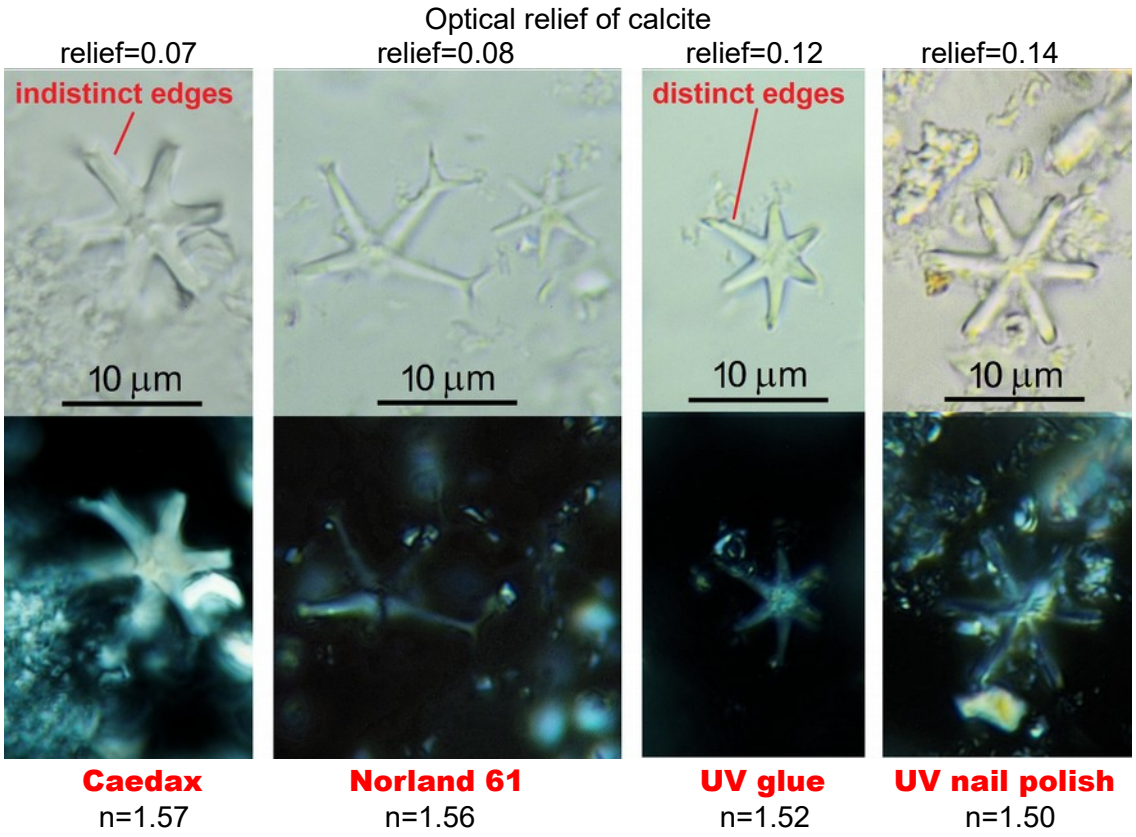


Figure 9. *Four preparations of the sample shown in Figure 6 to compare mounting media. The UV glue is Lazer Bond; the UV nail polish is Seche Ultra-V. Top row is plane polarized; bottom row is cross polarized. Photos were taken with a 100x immersion objective. The five specimens represent four species of Discoaster. From left to right they are D. surculus, D. pentaradiatus (broken specimen missing one arm), D. brouweri, D. asymmetricus, and another D. surculus.*

The slide in Figure 6 was prepared years ago with the now banned Caedax mounting medium. For comparison, I made new preparations of the same sample using the three UV-curing media considered in this article. For each preparation, about a half cubic mm of sample was dispersed in water on a cover slip and allowed to dry. A drop of mounting medium was placed on the slide and the cover slip was inverted (sample down) and lowered onto the slide. By mounting on the coverslip, the 0.17 mm coverslip thickness needed for optical correction with a high dry lens was preserved. Slides were warmed to reduce viscosity, then exposed to UV light to harden. These preparations are “whole rock smears”; by using the entire sediment, percent composition of components can be determined.

Figure 9 shows the results. As mounting medium R.I. decreases from 1.57, optical relief increases for calcite and decreases for diatoms – a “zero-sum game”. The UV-glue has a lower R.I. than Norland 61, giving greater calcite relief. The UV-nail polish has a still lower R.I.; this gives the greatest optical relief for calcite, but at the same time, it is the poorest relief for diatoms.

Example 2 Conclusions: A **higher R.I.** than Norland 61 would be better for small finely ornamented diatoms. A **lower R.I.** than Norland 61 would be better for calcite. When you are interested in both bio-silica and calcite components in the same sample, Norland 61 is a good choice. It is not the best medium for either, but is a reasonable compromise for each. If interested in only the calcite components, the Lazer Bond UV-curing glue provides better optical relief than Norland 61. The UV-curing nail polish has the best optical relief for calcite, but is too messy and tedious to recommend as a routine mountant.

Example 3: Vicuña Hair Fibers

The vicuña is a South American camel related to the llama. Vicuña wool is rare, expensive, and highly desirable. I obtained some thread clippings from a professional sewer/designer who had just completed a vicuña jacket for a client. Since these fibers were 1) spun into multi-fiber threads, and 2) subsequently woven into cloth, they have some mechanical damage such as folding, creasing, flattening, fiber splitting, and fiber breakage.

Vicuña fiber is characterized by a fine diameter which produces a lightweight soft fabric; by being hollow, which produces a warm fabric; by the closely spaced scale pattern (difficult to see in the light microscope); and by the guard hairs (often large in other species) being nearly the same diameter as the under coat. An on-line resource for vicuña fibers under the scanning electron microscope is Tillman and Tillman, 2006. Richard Howey did a series of *Micscape* articles on hair (Part 1, Part 2, Part 3); vicuña is illustrated in Part 3.

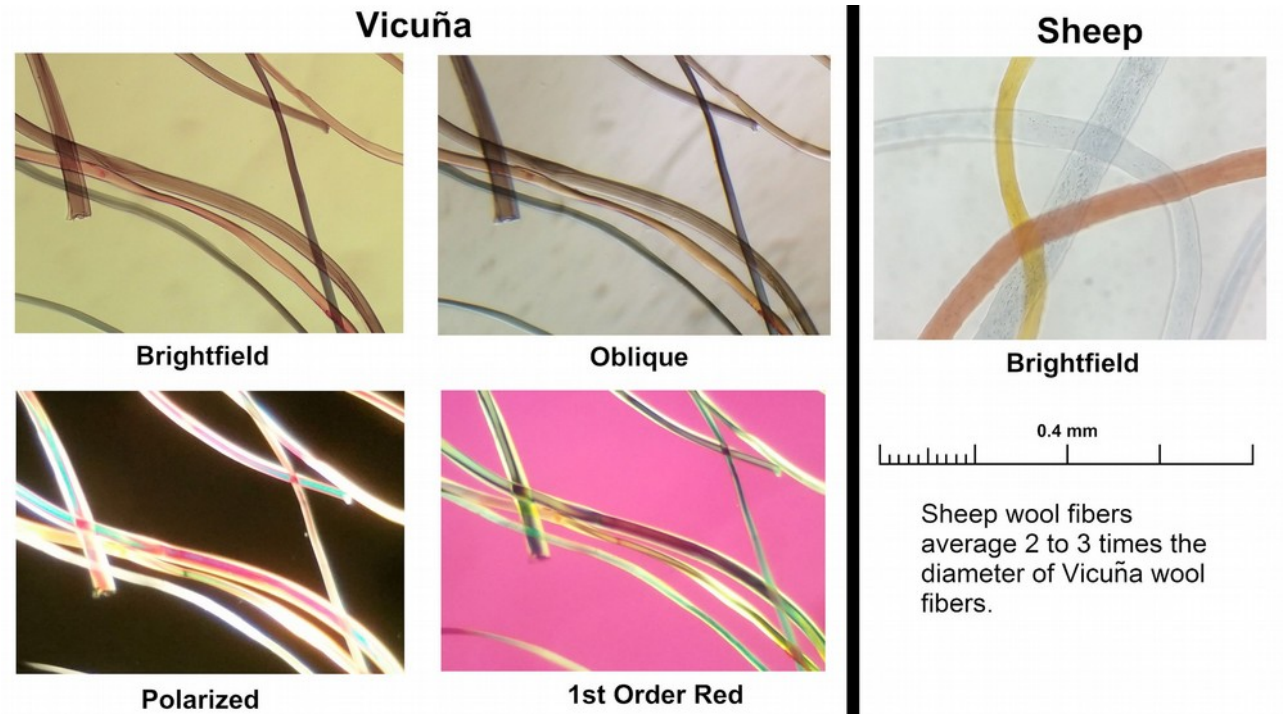


Figure 11. *Vicuña wool fibers compared to sheep wool fibers at the same magnification (20X objective). The vicuña wool fibers are mounted in UV-curing nail polish. The sheep wool fibers are a commercial educational mount from the 1950s of an unspecified variety of sheep wool in an unspecified mounting medium.*

This example is a difficult test for the three UV-curing media under consideration. Hair fibers are composed of the protein keratin and have an R.I. near n=1.55, which equates to low optical relief in all three UV media (Figure 12). I added a temporary mount in water to provide an image with some optical relief.

Example 3 Conclusions: None of the three UV-curing adhesives is a good choice for hair fibers; optical relief is too low. Color contrast shows the overall fiber, but surface details are subdued and difficult to make out (Figure 12). If you prepare a lot of hair fiber slides, and like the ease and convenience of UV-curing adhesive, it would be useful to test different brands to find one with a lower R.I.

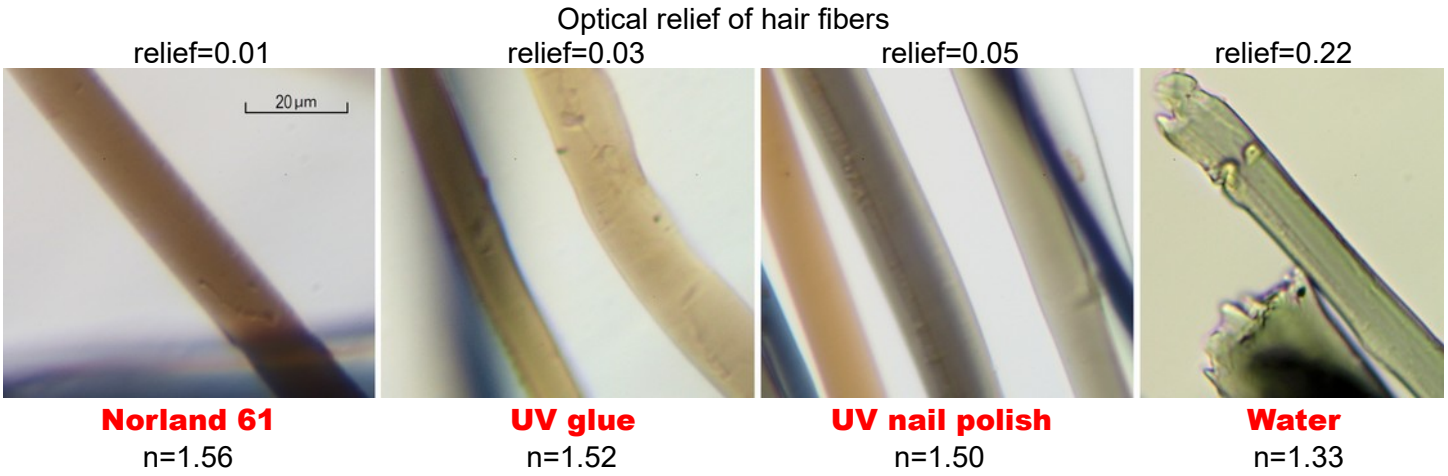


Figure 12. *Four preparations of vicuña wool fibers to compare mounting media. The UV glue is Lazer Bond; the UV nail polish is Seche Ultra-V. Photos were taken with a 40x objective in oblique light.*

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