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Taking pictures of tiny orchid flowers

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I recently obtained a specimen labeled *Notylia* sp. and wanted to get a positive identification for it, hence, needed a good photograph of the flower. The 5 mm flowers are very three-dimensional, so a challenge to shoot with regular photography.

Problem with regular photography — The issue is depth of field. The image is either tack sharp, but has paper-thin depth of field, or I can stop down, but the image gets degraded by diffraction. Notice, that one has to compensate f-stop settings in 1:1 macro, because the Airy discs forming the circles of confusions get enlarged along with the image. At 5:1, the effective maximum advantageous f-stop of f/32 at the image plane is reached when one sets the lens f-stop at 32/5+1 = 32/6 ~5.3! (See Ray. 2002. *Applied Photographic Optics*. Focal Press, Oxford for details).

Z-stacking. In this technique, one takes a series of images at consecutive image planes. The z-axis is the up and down axis of focus, hence the name of the technique. A computer program (I use HeliconFocus 5.1: www.heliconsoft.com) aligns the images (see below), then selects only the in-focus portion of each image, and combines those in-focus parts into one overall sharp image. It can be done with a regular camera and a sturdy tripod, but usually it is done on a stereomicroscope.

Photography on stereomicroscope — There are some considerations to be taken. The stereo effect is achieved by the images reaching each eye at an angle, usually 7 degrees. I.e., the optical axis and focus axis are not parallel. The image is only taken through one of those image channels. When focusing up and down the flower, the image moves from left to right. The images in the stack are not aligned. While the computer program can compensate for it a bit, it is better, when everything is neatly aligned to begin with. One can align the optical axis with the focus axis using a special attachment, the objective slider. There are some special photomacroscopes, like the Wild M40, which use a single optical pathway parallel to the focus axis.

Illumination — For best results, the illumination should be consistent and not change with altered focus. The light source can, therefore, not be attached to the lens of the microscope. The popular ring lights are not advisable. It would alter the illumination angle, and change the shadows on the image. For sharp images, they should be frozen, so flash photography is ideal.

Putting theory to work — I use a Zeiss Discovery V12 stereomicroscope (Figure 2) with a couple of planapochromatic lenses (0.63x, 1.5x). Those are the best lenses available. They have a flat image field important for projecting the image onto the flat image sensor, and have superior color correction, avoiding the dreaded blue and yellow color fringes at edges. The lens is mounted on an objective slider, meticulously aligned to my microscope. My digital SLR (Canon 5DmkII, 20 megapixel full-frame sensor) is tethered to my computer through which I release the shutter. I avoid touching the optical set-up and do not introduce unwanted vibrations.

A Canon MT-24EX twin head macroflash is attached to the camera, either directly or with a TTL synchro cable. The flash heads have a modified Garry Fong puffer pop-up flash diffuser attached to soften the light. The flash heads are positioned with two Wimberly PP100 Plamps, rather closely to the flower, so that the flash heads act as 'large light sources' with soft light, as opposed to point

light sources causing harsh shadows (See Hunter, Biver & Fuqua, 2007. Light: Science and Magic. Focal Press, Oxford for details). I modified the clasp end by gluing a flash mount adapter with epoxy to one of the segments; the head portion can now be changed for different purposes, for instance to hold small epiphytes in front of background for regular photography.

For each shot, 13–18 images were taken, changing focus by tiny, constant increments. I do it manually, but it could also be done with a motorized focus stepper. One starts at the bottom of the object, and focuses the microscope up, against gravity. This ensures that the microscope moves, and does not remain in place due to friction between body of the microscope and the stand, which could cause irregular focus increments.

The CR2 RAW files were run through HeliconFocus 5.1, the process taking about 15 minutes each. Then the resulting z-stacked image was minimally cleaned up in Photoshop.

It may seem like a lot of work just for a tiny flower. Once one considers the effort that goes into producing the bloom, it is only doing justice to those little jewels. I posted those images on Orchidboard, and 20 minutes later Ronald Hanko provided the name for my plant: *Zygostates pellucida*.

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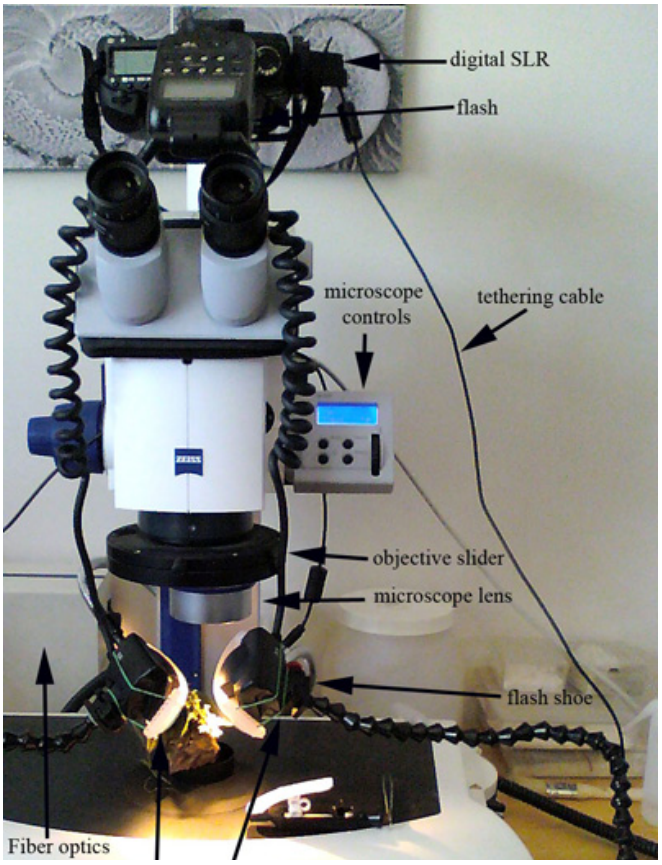

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