
ORCHID DIGEST
July, Aug., Sept. 2013
Vol. 77-3

ORCHID DIGEST

VOLUME 77, NO. 3—July, August, September 2013

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Front cover — 9607: *Oberonia* species: 33 image z-stack on Zeiss Discover V12 with Zeiss planapochromatic 0.63x lens. See Mr. Geiger's article on page 112. ©Daniel Geiger

Back cover — *Bulbophyllum flavescens*. ©Daniel Geiger

IMAGING SMALL ORCHID FLOWERS USING VISIBLE LIGHT

DANIEL L. GEIGER

Introduction

ORCHIDS ARE ONE OF THE LARGEST families of flowering plants with an estimated 30,000 species, with additional ones being discovered on a regular basis. Documenting biodiversity and range of morphology can be accomplished by various collecting techniques. Given that all orchids are CITES protected, non-destructive documentation, such as photography, has an important place in the documentary efforts. Additionally, many horticulturists enjoy taking images of their flowers. The majority of flowers are small and small objects are more challenging to photograph.

In this article, I will provide an overview of techniques for imaging small orchid flowers. Successful photography is based on a thorough understanding of the working principles. Accordingly, I will supplement simple rules of thumb with detailed explanations of why those rules are in place. It will require diving into principles of optics and physics. I have strived to keep the technical portion as easy to follow as possible, while still being accurate, and never losing touch with the practice of imaging flowers.

Camera Systems

There is a myriad of camera systems on the market place, ranging from cell phones to compact cameras and digital single lens reflex (SLR) cameras. Additionally there are some more esoteric approaches to imaging orchids, which will be dealt with on a more peripheral level: large format photography, photomicrography, and z-stacking. For most people, the former category is the desirable one; hence, let us examine the advantages and disadvantages of each option.

Cell phones are ubiquitous, and easy to carry around. Image quality is limited and for close-up imaging they are almost useless. Compact cameras are more versatile, and can deliver reasonable results for close-up imaging. Adjustments of variables (f-stop, exposure) are rather limited, and they are generally not modular, so cannot be adapted for special purposes.

SLR cameras are possibly the most popular cameras for serious photographers. The modularity permits the photographer to adapt the imaging system to a large number of circumstances. Close-up and true macro photography (magnification of >1:1) are easily achieved with SLR cameras. When selecting a particular camera system for plant photography, Table 1 lists the more common features available on consumer cameras, and compares them to the desirable attributes that professional (or prosumer) cameras offer. The most important features are: large sensor (24 × 36 mm), RAW file capture, and mirror lock-up.

What is even more important is the selection of the lenses. The general perception that the number of megapixels of the camera is paramount for image quality is wholly mistaken. The lens is the weak link. Accordingly, when selecting a camera system, first look at the lenses and consider purchasing the best lens you can buy. What is left over in the budget can be spent on the camera body. Table 2 compares various lens attributes and their advantages and disadvantages.

For scientific photography image distortion is an important factor. For this reason, avoid zoom lenses, which have inherently more distortion, and distortion varies with focal length chosen; hence, they are unsuitable for documentary reproduction photography. Choose high quality, fix-focal (= prime) lenses. Choose

Consumer	Professional	Why?
Small sensor	Full size sensor 24 × 36 mm	Larger pixels, better signal to noise ratio, wide angle lenses.
Fewer pixels	More pixels	Larger prints, partial enlargements
.jpg	RAW-NEF-CR2	Better processing in Photoshop
LCD viewfinder	Optical viewfinder	Clearer image
Smaller battery	Larger battery	More pictures
Only one shutter release	Also high-format shutter	Easier to hold
No mirror lock-up	With mirror lock-up	Reduced vibrations = sharper images
Fixed focusing screen	Interchangeable focusing screens	Use of manual focus lenses, macro-/ micro-photography
Less sealed	Better sealed	Cold weather, tropics reliability
Lightweight	Heavy	[no free lunch]

Table 1. Comparison of consumer and prosumer/professional SLR camera attributes relevant for imaging small plant flowers. Other attributes are mostly gimmicks, irrelevant for plant photography (frames per seconds, custom function, movies, second curtain synchronization).

Attribute	Advantages	Disadvantages
APC lens	Light, cheap	APC cameras only, poor quality
Full-frame lens	Better quality	Heavier, more expensive
Zoom lens	Versatile	Poor quality images
Prime lens [="fix-focal"]	Better quality images	Limited use
Autofocus	Convenient	Close-up problems, construction tolerances
Manual focus	Tight manufacturing, deliberate	No snap shots, focusing screen
Standard line	Cheap	Poor quality glass: distortions, aberrations
Premium line	Good glass (ED, aspherical, larger opening)	More expensive, heavier
Third party	- Very cheap and poor quality (e.g., Zenith) - Cheaper than big brand at same quality (e.g., Tokina, Sigma) - Expensive but top notch (e.g., Zeiss)	

Table 2. Comparison of various lens types, their advantages and disadvantages. Best images are obtained with manual prime lenses from Zeiss (full frame). Note: Image quality limited by quality of lens, not by number of pixels.

dedicated macro lenses as opposed to using extension rings on normal lenses. While both lenses (macro and normal) can be focused from infinity to close focus, normal lenses are optimized for various color errors and optimal resolution at close to infinity, while macro lenses are optimized in the range of 1:10–1:2 reproduction ratio.

Best Camera?

There is no such thing. There are good choices for the task at hand, and there are good choices for a person. For the latter, consider what is important for you, and what you can live without. First consider the lenses that will permit you to take the images you want to take, then consider the camera body that can be attached to that lens. Also try to think ahead: what else should this camera be able to do? Do you like to take movies? Are you into sports or architecture? Last but not least, there is the budget question. I would strongly suggest to invest in good lenses, and to use a lesser camera body with that lens, than the other way around. Also notice, that camera models are much more frequently updated than lenses.

Improve Your Images

We all have images that we are not happy about, myself included. How do you improve your flower images? In the following I will detail a few simple approaches.

✔ Use a tripod. This even applies for cell phone users: yes, put a cell phone on a tripod. Better compositions will result, and images will be sharper. If you move the camera during exposure by one pixel, it will cut resolution in half! Pixels are approximately 4–10 µm = 0.004–0.010 mm wide. Do you really think you keep the camera from moving by that much while the image is taken? Think again. Whether a copy stand or a tripod is used makes little difference. For fine adjustment in x-and z-axis, consider a macro-focusing stage, which are offered by a number of vendors (Adorama, Novoflex, Really-RightStuff; Cognisys: see also below for z-stacking).

✔ Use a shutter release. A shutter release will prevent you from moving the camera by depressing its shutter directly. If you don't have a remote shutter release cable, then the self-timer can be used for the same purpose.

✔ Use a reflector. One of the hallmarks of beginner's images are strong shadows on the image, particularly on flash images. Use a piece of white cardboard, some white foam board, or dedicated photoreflectors (such as the Photoflex Litedisc) opposite the light source to brighten up the shadows. It will immediately notch your images up by a category or two, because the illumination of the flower will be much more even.

✔ Take more pictures. Most beginners are stingy with pictures. Now in the digital age, there is no reason for not taking more pictures. Experiment. Try something different. Hold the camera in high-format, or even upside down if it has a built-in flash. Hold the reflector in different positions and at different angles. Change the camera angle. Change the framing. Try various f-stops. Focus on different parts of the flower. I usually take around 30–50 images per framing. After all these images are taken, edit ruthlessly. Compare the various images, find differences, form an opinion, delete all those that are inferior. Usually I end up with 3–4 keepers.

✔ Read. Photography is first a craft, then in second place an art. Only once one has mastered the craft, can one apply that knowledge and make art with it. This article cannot replace a more in-depth exposure to reading material. I recommend the following volumes:

- Ray (2002) *Applied Photographic Optics*. As mentioned above, the lens is the weakest link in the photographic chain. Understanding how to choose the appropriate lens and its settings is crucial for successful photography. This book provides a thorough explanation of anything related to optics in photography. It is supported by all mathematical derivations, but they can be skipped.
- Hunter et al. (2007). *Light: Science and Magic*. This volume explains lighting techniques. It emphasizes a basic understanding rather than providing recipes for a given situation.
- Freeman (2009). *Perfect Exposure*. Capturing the light is paramount in photography. Despite lots of automatic functions on modern cameras, understanding the assumptions of those auto-exposure functions enables the photographer to recognize situation that will fool the camera, and provides insights of how to correct them.

Lighting

Whatever gets the job done is suitable (Fig. 1: *Trichoceros muralis*, Fig. 2: *Masdevallia medinae*, Fig. 3: *Dryadella zebrina*). Continuous light such as photo-lamps, or fiber optics light sources permit to immediately see the result from any adjustments made. However, they usually have relatively low light output and generate a lot of heat, which leads to long exposure times and can negatively affect live plants. Both may lead to the plant moving during exposure and lack of sharpness. When multiple lamps are used, it still is important that they all have the same color temperature. Non-standard color temperature can be adjusted either with a custom white-balance on the camera, or during RAW image conversion. However, differences in color temperature from different light sources cannot be adjusted. It is easiest to only use one type of light source for any given image.

Flashes produce short light pulses of standardized color temperature that almost always ensure crisp images, but adjustments in position of the flash(es) can

Fig. 1. *Trichoceros muralis*. Lighting is key to good photography. The hairs on the petals are clearly visible, which requires side light. The shiny top bud has no blown out highlights, which is accomplished by using a light diffuser, and careful adjustment of the flash power. Slight overexposure can be rescued during RAW conversion.

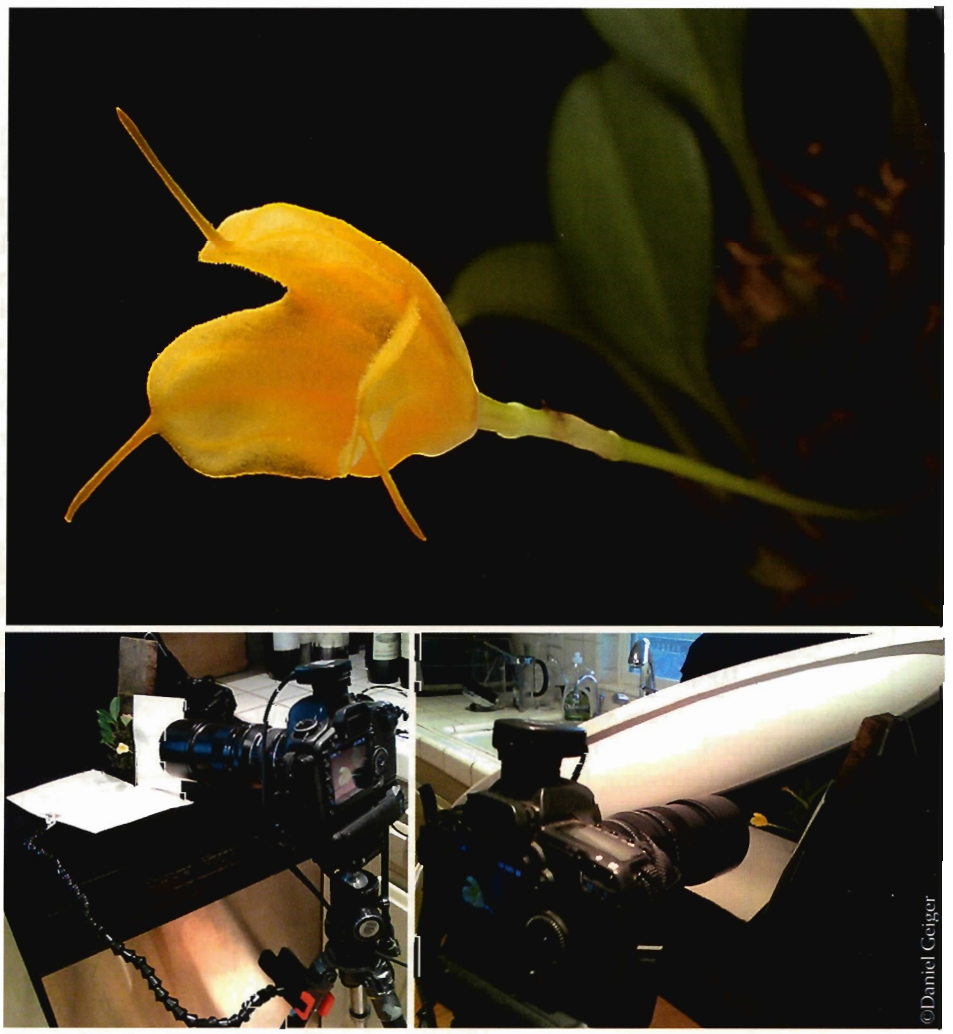

Fig. 2. *Masdevallia medinae*. Flowers with deep recesses, such as this *Masdevallia medinae*, often have strong shadows inside the cup. Brightening cardboards were placed below and to the right of the flower. A diffusor screen is placed at an angle over the plant, with the flash directed into the cup.

only be judged after a test-image has been taken. For close-up and macro-photography shoe mounted flashes are useless; the flashes (or heads in case of macro flashes) are positioned off camera and are connected to the camera using cables. FlashZebra (www.flashzebra.com) makes a number of flash synchronization cables not available from major manufacturers.

Ring flashes are often suggested for macro photography. There are two problems with them. For one, the even illumination they generate robs the specimens of their three-dimensional aspects. Much of the interest in orchid flowers is precisely that three dimensionality, for which reason shadows and highlights can help to visualize that aspect. Second, ring flashes are attached to the front of the lens without any options for adjustments. At higher magnifications the subject is very close to the lens, while the ring flash emits light at a fixed angle. In that case, the background is illuminated, leaving the flower literally in the dark. I much prefer dual head macro flashes, because I can remove the heads from the mounting ring on the lens, and place them anywhere I like to produce the right shadows and highlights and direct the light onto the flowers. I adjust position, distance, direction, strength, and ratio of flash power to generate the light I want.

Light modifiers are crucial, (Fig. 4). The simplest reflectors are white pieces of cardboard and aluminum foil. Avoid white paper, particularly with flash photography, because the UV brighteners in the paper produce a blue cast in the image. Less harsh and more diffuse lighting can be achieved by pointing the light source away from the flower at a reflector. For this purpose consider in addition to cardboard and aluminum foil also white ceramic bowls of various sizes, which generate exquisite soft lighting. Diffusors may occasionally be helpful, such as the Photoflex Litediscs.

Exposure

As a general guideline, expose to the right. It refers to the histogram of the image, which should rather be shifted to the right (highlights) than to the left (shadows). The sensor of the camera usually can capture a greater range of light intensity values than are dis-

Fig. 3. *Dryadella zebrina*. Comparison of different lighting options (*Dryadella zebrina*). **Top right.** Image taken with compact camera with built-in flash. Notice very harsh directional lighting. **Left.** Dual bounce lighting. One flash head was directed into a white cereal bowl above, acting like an umbrella in portrait photography, the other flash was directed onto a white cardboard below. Notice, no light hit the plant directly, resulting in very even illumination. **Bottom right.** Dual flash-heads with diffusers directed at the plant. Notice stronger shadows on leaves, but also better rendition of the surface pustules on the ventral sepals.

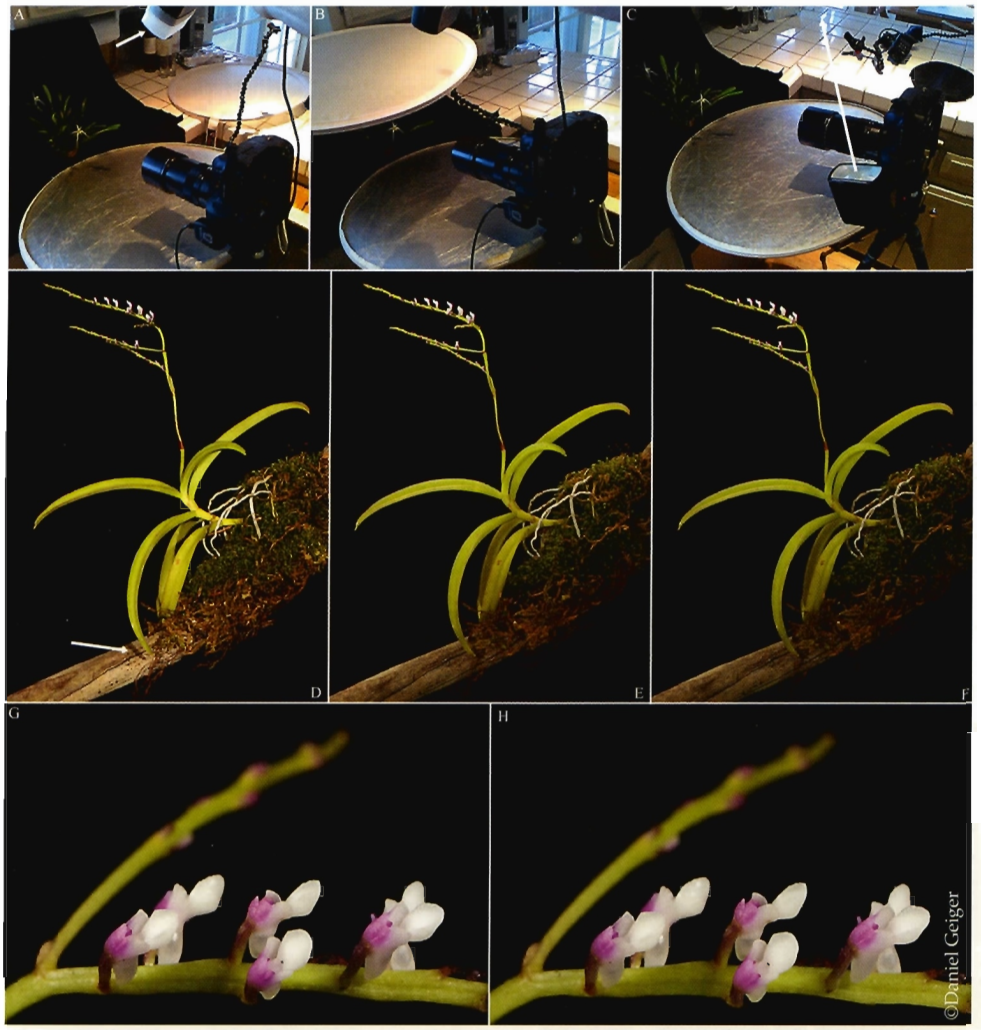

Fig. 4. Soft light. **A–C.** Three ways to soften light. *Dendrobium aratriferum* (*Diplocaulobium*). **A.** Flash with Omnibounce diffuser attachment (arrow). **B.** Flashing through LiteDisc diffuser. **C.** Indirect flash against white ceiling; arrow indicates direction of light towards ceiling. Silver reflector is used in all as counter balance to top illumination. Notice that the "studio" is a simple kitchen counter. **D–F.** *Schoenorchis gemmata* habitus. **D.** With Omnibounce diffusor. Notice some strong shadows of leaf on twig (arrow). **E.** LiteDisk Diffuser. **F.** Indirect flash to ceiling. **G–H.** *Schoenorchis gemmata* flowers. **G.** Double overhead flash-heads cause some shadows on underside of rachis. **H.** Placing white cardboard underneath alleviates those shadows.

played on the built-in LCD screen. The full range of intensity values is encoded in the RAW file. The signal to noise ratio is better in the highlights than in the shadows. Accordingly, some select "overexposed" areas can be adjusted during RAW image conversion. Brightening very dark areas leads to mottled appearance of the image.

Focus and Depth of Field

Focus is always a geometric plane at right angle to the lens axis. The area that appears sharp can be varied by opening and closing the diaphragm or f-stop. Sharpness in normal photography extends 1/3 to the front of the focal plane and 2/3 to the back of the focal plane. In macro photography, this ratio shifts to 1/2 and 1/2 to front and back. With some lenses the focal plane also shifts after stopping down, which is referred to as focus creep. To check for it, use the depth of field preview button (if available).

The photographer has to make a conscious decision where to place the focal plane. With a single point of interest, the image can be taken from any angle. With two points of interest the geometric line connecting those two points must remain parallel to the sensor plane, and camera position can only be rotated around that line (Fig. 6). With three points of interest the direction from which the image is taken is fixed. With more than three points the photographer has to make a decision of which points are most important, which may appear sharp due to depth of field, and which have to be accepted to be shown blurry.

F-stop and Diffraction Blur

In macro photography limited depth of field is an issue. It can be increased by stopping down the lens (= using a higher f-stop number). However, at a certain point, stopping down the lens will introduce image blur due to diffraction (= bending of light at an edge). Diffraction will cause a point to be imaged as an Airy disc (Fig. 5), and the diameter of the f-stop increases with smaller f-stops (= higher number). Two factors affect the largest f-stop number (f_{max}) that should be set on a given image: 1) image magnification from object to sensor. Magnification is the ratio of linear dimension imaged to linear dimension of sensor (or film). 2) image magnification from native sensor image to final print image. Most people will consider an image area as blurred if the Airy-discs of two points separated by 1/30 mm (= circle of confusion) can no longer be separated. This happens above f/32 for an image taken at infinity distance with a full-size sensor (24 × 36 mm) printed to 8 × 10" and viewed at arm's length (~2' = 60 cm). Let us examine the above two factors accordingly.

1) Due to the spreading of the light when the image is

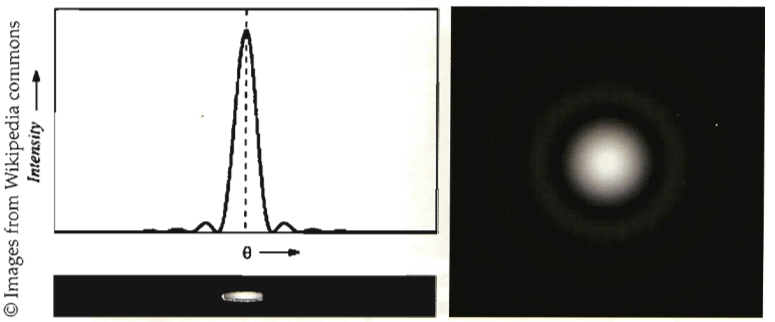

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Fig. 5. Diffraction pattern (Airy disc) of an image point. Intensity distribution and two-dimensional pattern.

Fig. 6. *Stelis hallii*. The focal plane was intentionally placed in the stigma of the right front flower, and the front bottom left bud. The other flowers are out of focus.

enlarged on the sensor, the effective f-stop (f_{eff}) increases with image magnification (M) according to $f_{eff} = f_{set} \times (M + 1)$. To obtain the maximum advantageous f-stop for a given magnification, use $f_{set} = 32 / (M+1)$, where 32 is the desired maximum advantageous f-stop of f/32. Table 3 provides the f-stop that needs to be set on the lens to attain the desired effective f-stop for magnifications from 1:1 to 5:1 in full magnification and f-stop increments. While most macro lenses stop down to f/22, for an effective f-stop of 32, at life-size (1:1) only f/16 should be set, while f/5.3 is the maximum advantageous f-stop at 5:1.

2) Perceived sharpness is related to magnification of the image. Take any digital image, reduce its size to that of a stamp, and it will look sharp. Increase the size of the same image to 1600% and it will look blurry. This demonstrates that perceived sharpness depends upon final magnification of the image. Table 4 gives the maximum advantageous f-stop for a number of sensor and print sizes.

Depth of field becomes extraordinarily shallow in macro photography, particularly when respecting fmax. Table 5 provides f-stop, effective f-stop, depth of field, and step size for z-stacking (= 70% of depth of field) for magnifications of 1:10 to 5:1 at various f-stops. Table 5 also highlights in green the suitable f-stops at those magnifications. Many macrolenses have a largest f-stop of f/2.8, which was chosen here as the starting point for the f-stop scale. Optimum sharpness of images is obtained at 1–2 f-stops smaller than fully open. Accordingly, the first two f-stops are considered unsuitable for optimum photography. F_{eff} should be smaller than f/32, which imposes the upper limit on the f-stop to be set on the lens. As a consequence, at higher magnifications (4–5:1), only a single f-stop (f/5.6) remains suitable! At 5:1 depth of field is 0.081 mm = 81 µm.

f_{eff}	1:1	2:1	3:1	4:1	5:1
2					
2.8	1.4				
4	2	1.3			
5.6	2.8	2.6	1.4		
8	4	2.7	2	1.6	1.3
11	5.6	3.6	2.8	2.2	1.8
16	8	5.3	4	3.2	2.7
22	11	7.3	5.6	4.4	3.7
32	16	10.6	8	6.4	5.3

Table 3. Effective f-stop (f_{eff}) and f-stop to be set on lens at magnifications from 1:1 to 5:1.

	Print Size (inches)			Print Size (inches)		
Sensor size	2 × 2.5	4 × 5	8 × 10	2 × 2.5	4 × 5	8 × 10
	Absolute values of f_{max}			Change from f/32 on 24 × 36 mm sensor to 8 × 10″ print		
12 × 18 mm = 4/3 size	45	32	22	+1	0	-1
APS-C flavors	45–64	32–45	22–32	+1 – +2	0 – +1	-1 – 0
24 × 36 mm	64	45	32	+2	+1	0
645	90	64	45	+3	+2	+1
4 × 5″	128	45	64	+4	+3	+2

Table 4. Absolute f_{max} values and relative change from f/32 with full size sensor (24 × 36 mm) and reproduced at 8 × 10″, for given sensor/film size and final reproduction size to avoid diffraction blur on final image.

Greater Than Life Size Imaging

Most macro lenses permit magnification to either 1:2 (half life-size) or 1:1 (life-size). Greater than life-size images can be obtained by a number of means. Extension rings are possibly the easiest. Next are dedicated lenses that are designed specifically for greater than life size imaging, such as the Canon MPE 65 mm or the Zeiss Luminar series. Normal lenses can be mounted reversed on extension rings or bellows, and last but not least a tele lens can be mounted normal on the camera body, and a normal lens is attached reversed on the tele lens (= lens stacking). I will not further discuss the latter two options, but it may provide the inclined reader with a starting point for further investigations. Above about 10:1 magnification, the use of SLR systems becomes rather challenging. Issues are very dark viewfinder, almost unavoidable diffraction blur (f_{max} is 3.0), and lack of stability of the entire apparatus. I prefer to then switch to a stereomicroscope, which is designed to be used at magnifications of 5–200×.

Larger Formats

There are cameras with larger sensors or using larger pieces of film. Medium format cameras (645 format and similar) can be fitted with very expensive digital backs. The larger image area permits to use larger f-stops for any given final reproduction size (Table 4). From the optical perspective, medium format cameras are oversized SLRs.

Large format cameras generally still use film in sizes of 4 × 5″ to 20 × 24″ and beyond. There are some digital options, but they are rather cumbersome. Large format cameras differ from SLRs in that the lens as well as the film holder can be rotated both horizontally as well as vertically, and can also be moved side by side and up and down. Those movements permit to place the focal plane independently of camera angle. Those systems are tools for optical perfectionists, and still produced unsurpassed quality images. The operation is very tedious because nothing is automatic. A simple snap shot takes about 10–15 minutes to set up, a more complicated macroshot easily over an hour. Taking such cameras into the field requires the photographer to be in good physical shape; my basic pack weighs around 40–50 lb, without any food or water. Differences in placement of the focal plane can be visible even in small reproductions, but the greater resolution can only be appreciated in large gallery-style prints (Fig. 7).

Z-Stacking

Due to the narrow depth of field and the very three-dimensional nature of orchid flowers, other means have to be adduced to render small flowers sharp in their entirety. This process is referred to as z-stacking. The z-axis is the optical axis, where x and y are in the plane of the image and refer to left-right and up-down. In z-stacking multiple images in sequential focal planes are captured. Those images are computer processed, where a dedicated program chooses the sharp portions of each frame and unites those sharp portions of all frames into a single image that is sharp from front to back. Z-stacking can be carried out with regular cameras as well as with microscopes.

Optical Requirements

The optical axis should be parallel to the z-axis. With SLR systems this is a non-issue. Stereomicroscopes can be problematic because the stereo effect is obtained because the optical axes are at 7.5° angles to the up-down focus axis. The camera system only captures the information from one of the light paths. While focusing up and down the image will move left/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.

You can check whether your microscope has a single optical axis or two by looking through one ocular only, focus up and down. If the image moves from side to side, your microscope has the optical axes at an angle, if it stays stationary, you the optical axis is parallel to

Fig. 7. Large format photography. A–B. Two macro set-ups with a 4 × 5" large format camera. **A)** Some yellow button mushroom. **B)** An orange coral fungus. Notice in A that the film plane on the right is not parallel to the lens plane on the left. The cardboard reflector is attached with blue masking tape to the compendium shade. B shows a "belly shot", with camera inverted hanging down from tripod. The same principles for illumination apply (reflectors, diffusors). **C)** *Epipactis gigantea* inflorescence at greater than life-size taken with an Arca-Swiss 4 × 5" camera, a 180 mm ApoMacroSironar, and flash, on Provia 100F slide film.

the z-axis.

Choose f-stop for maximum sharpness, not depth of field. Because the computer algorithm will generate the increased depth of field, the base images should show maximum sharpness. Accordingly, the f-stop set on the lens should be 1–2 f-stops down from fully open; on a stereomicroscope the f-stop (if available) should be fully open.

Change focal planes by moving camera. In normal photography, the focus ring is turned to adjust focus. In z-stacking, the focus of the lens is not changed after initial set-up. Autofocus has to be turned off. Instead, the entire camera set-up is moved relative to the flower. For this purpose a focusing rail is critically important (Adorama, ReallyRightStuff, Cognisys: Fig. 8).

Steps should be even and result in overlapping sharp areas. The computer programs assume that the images are evenly spaced between frames. To achieve optimum results, the steps should be about 70% of depth of field (see Table 5). While respectable images can be obtained by manually moving the focusing rail, high precision is afforded by a motorized and microprocessor/computer controlled rail, such as the Cognisys' StackShot. When z-stacking looking from above as on a photographic copystand or when using a stereomicroscope, one starts at the bottom of the object, and moves the optical system up, against gravity. This ensures that the optics moves, and does not remain in

Fig. 8. SLR z-stacking set-up with computer controlled, motorized focusing rail (Cognysis Stackshot).

M	f-stop	F_{eff}	DOF (mm)	70% DOF (mm)
1	2.8	5.6	0.336	0.235
1	4	8	0.480	0.336
1	5.6	11.2	0.672	0.470
1	8	16	0.960	0.672
1	11	22	1.320	0.924
1	16	32	1.920	1.344
1	22	44	2.640	1.848
2	2.8	8.4	0.126	0.088
2	4	12	0.180	0.126
2	5.6	16.8	0.252	0.176
2	8	24	0.360	0.252
2	11	33	0.495	0.347
2	16	48	0.720	0.504
2	22	66	0.990	0.693
3	2.8	11.2	0.075	0.052
3	4	16	0.107	0.075
3	5.6	22.4	0.149	0.105
3	8	32	0.213	0.149
3	11	44	0.293	0.205
3	16	64	0.427	0.299
3	22	88	0.587	0.411
4	2.8	14	0.053	0.037
4	4	20	0.075	0.053
4	5.6	28	0.105	0.074
4	8	40	0.150	0.105
4	11	55	0.206	0.144
4	16	80	0.300	0.210
4	22	110	0.413	0.289
5	2.8	16.8	0.040	0.028
5	4	24	0.058	0.040
5	5.6	33.6	0.081	0.056
5	8	48	0.115	0.081
5	11	66	0.158	0.111
5	16	96	0.230	0.161
5	22	132	0.317	0.222

M	f-stop	F_{eff}	DOF (mm)	70% DOF (mm)
0.5	2.8	4.2	1.008	0.706
0.5	4	6	1.440	1.008
0.5	5.6	8.4	2.016	1.411
0.5	8	12	2.880	2.016
0.5	11	16.5	3.960	2.772
0.5	16	24	5.760	4.032
0.5	22	33	7.920	5.544
0.33	2.8	3.7	2.052	1.436
0.33	4	5.3	2.931	2.052
0.33	5.6	7.4	4.104	2.873
0.33	8	10.6	5.862	4.104
0.33	11	14.6	8.061	5.642
0.33	16	21.3	11.725	8.207
0.33	22	29.3	16.121	11.285
0.25	2.8	3.5	3.360	2.352
0.25	4	5	4.800	3.360
0.25	5.6	7	6.720	4.704
0.25	8	10	9.600	6.720
0.25	11	13.8	13.200	9.240
0.25	16	20	19.200	13.440
0.25	22	27.5	26.400	18.480
0.2	2.8	3.4	5.040	3.528
0.2	4	4.8	7.200	5.040
0.2	5.6	6.7	10.080	7.056
0.2	8	9.6	14.400	10.080
0.2	11	13.2	19.800	13.860
0.2	16	19.2	28.800	20.160
0.2	22	26.4	39.600	27.720
0.1	2.8	3.08	18.480	12.936
0.1	4	4.4	26.400	18.480
0.1	5.6	6.16	36.960	25.872
0.1	8	8.8	52.800	36.960
0.1	11	12.1	72.600	50.820
0.1	16	17.6	105.600	73.920
0.1	22	24.2	145.200	101.640

Table 5. Calculation of effective f-stop (f_{eff}), depth of field (DOF), and step-size for z-stacking (= 70% of depth of field), for various magnifications and f-stops. The left column is for magnifications of greater than life size (to 5:1), the right column for magnification smaller than life-size (to 1:10). DOF was calculated as 2 × f-stop × c × ((M+1)/(M^2)), where c is the circle of confusion taken as 0.03 mm. Notice, that depth of field is independent of focal length, and only depends upon magnification (M) and f-stop.

The green area encompasses those f-stop settings that maximize sharpness without causing diffraction blur.

place due to friction between body of the camera and the stand or focusing rail, which could cause irregular focus increments.

Clean the optics and sensor (Fig. 9). Dirt on the optic and particularly the sensor are always in focus. Those dust particles will be retained from each of the captured images. Because even with best care, the images are not perfectly aligned to one another, those dust particles will appear as meandering paths of dark specks. Those have to be digitally cleaned-up either in the stacking software package or in Photoshop. To minimize the amount of time required for clean-up, it is best to start off with clean images. Some cameras have a dust-delete function, which may be helpful.

Illumination Requirements

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 camera or 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. The illumination should be even across all exposures, for which reason manual exposure with fixed time or with manual setting of flash power is advantageous. Stacking software can compensate to a certain extent for uneven exposures across frames, but the fewer adjustments that have to be carried out, the better.

Stacking Software

Once the images have been acquired, they have to be processed by the stacking software. The two most popular packages are HeliconFocus and Zerene Stacker (Fig. 10). Although Photoshop has a z-stacking function, CS5 results from microscope images are unusable. The newer Photoshop CS6 seems to be improved, but still is inferior to the dedicated packages (Burkholder, 2012).

The two dedicated packages both have their virtues. Both offer free limited demo version downloads and are rather inexpensive. Differences are in computer platform they run, file types they accept, proprietary stacking algorithms, and interface with external hardware such as the Cognysis motorized focusing rail (Fig. 8). Serious z-stackers will eventually own both programs. The time to analyze an image stack depends on a number of factors such as number and size of images, file format, and computer speed. A short jpeg stack may take just a minute or two, while a large CR2 stack may take 15 minutes or longer.

Occasionally, unsightly halos appear at the eges of flower parts. Some can be removed in Photoshop, or using the editing options in the stacking software. In cases where the halos intrude into other parts of the flower, it is better to do recursive z-stacking. In the example in Fig. 11, the complete stack of 19 images was subdivided into one stack with 6 images for the rachis of the inflorescence, and one stack with 13 images

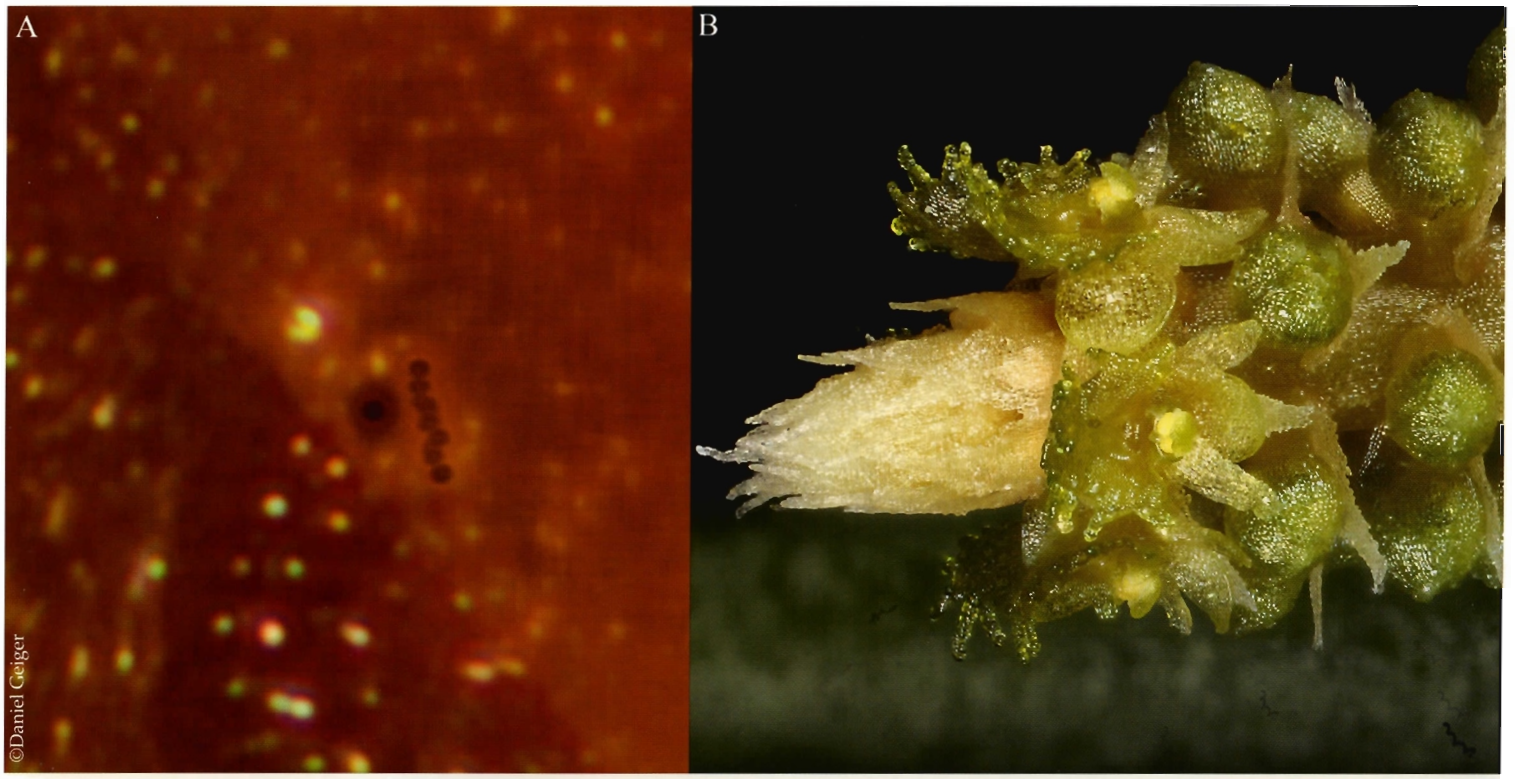

Fig. 9. Z-stacking and dirt. **A.** Linear streak of dirt particles from about 12-images-stack on stereomicroscope with rack and pinion gear. Strong partial enlargement of image. **B.** Spiral dirt tracks from 74-images-stack taken on motorized focusing rail with helical drive. *Oberonia cavaleriei* Finet, 1908, usually wrongly referred to as *O. myosurus* [= *Phreatia matthewsii*].

Fig. 10. My Zeiss Discovery V20 microscope with mounted microscope camera (Zeiss Axiocam HRc). Notice that the lens is not in the middle of the microscope, so that the optical axis and the z-axis are parallel. *Oberonia* cf. *mucronata* is on the stage. The computer screens show the Zeiss Zen Blue 2012 microscope control software for image acquisition, and the Zerene Stacker for better image processing of deeper stacks.

for the flowers only. In a second step, the two resultant images from the partial stacks were stacked, resulting in much reduced halos around the lip of the two flowers.

The "Studio"

One of the advantages of photographing small flowers is that the requirements for the "studio" are much simpler. I take most of my images of plants in my collection on my kitchen counter (Fig. 8). The backdrop is a cutting board with black cloth draped over it. I position plants with anything I have handy: photo equipment not used, food cans, bottles, clamps.

SLR Equipment

I will briefly describe the equipment I use and why. This may provide some insights about how to think about taking pictures of small orchid flowers and how to think about your own equipment. As indicated above, this is not the objectively best set-up, but works well for me.

The camera is a Canon 5d mkII, a full frame camera, with mirror lock-up and live view permitting partial enlargements (5×, 10×) to check fine focus on portions of the image, on which I can also mount my old Contax/Yashica Zeiss lenses with an adapter. Because

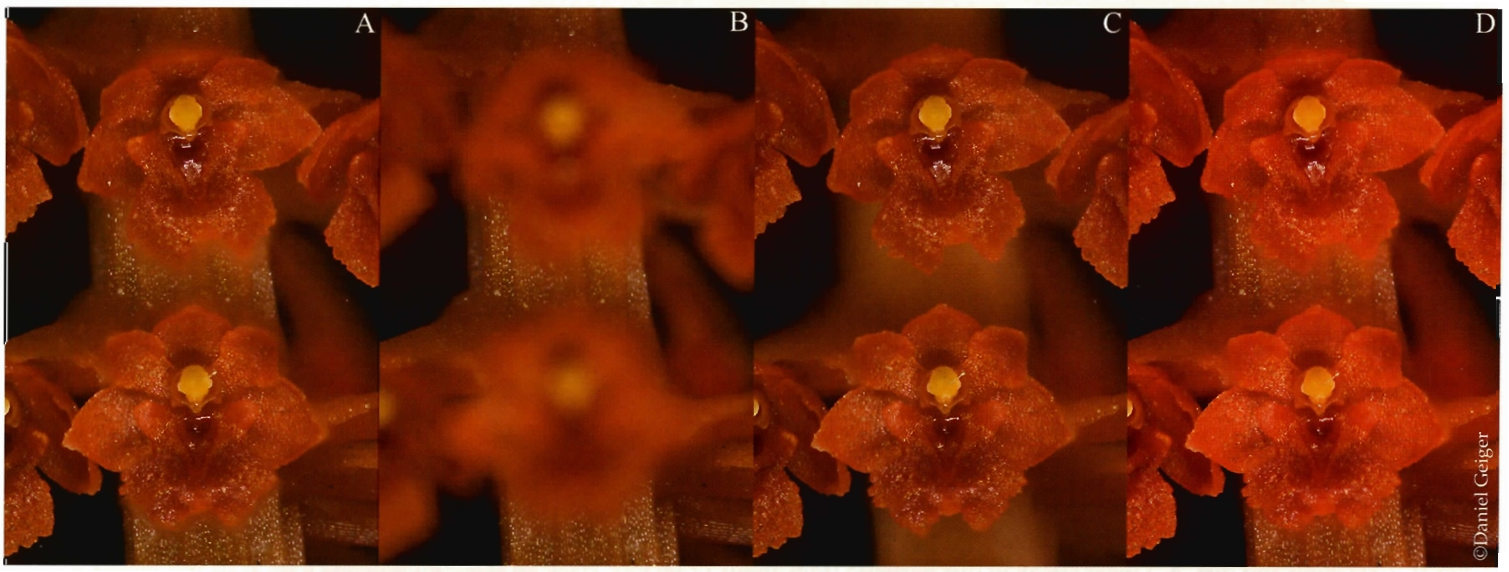

Fig. 11. Recursive z-stacking. *Oberonia leytensis*. **A.** Z-stack of 19 images. Notice strong halos around lip of upper flower. **B.** Partial z-stack of 6 images of rachis. **C.** Partial z-stack of 13 images for flower. **D.** Secondary z-stack of the B and C. Notice much reduced halos around lips.

I use manual focus lenses, I use a Haoda matt focusing screen to examine the aerial image. I have mostly Zeiss manual focus lenses from 16 mm fisheye to a 100–300 mm zoom, and also a Canon 300 mm f/2.8 IS. I use Zeiss lenses for their unparalleled quality. It comes at a price, but it is worth it for me. For macro photography I mainly use the Zeiss Makroplanar 100 mm ZE, and the Canon MPE 65 mm. The former covers from infinity to 1:2; with extension rings it can provide magnifications above 1:1. The latter covers the range of 1:1 to 5:1. A teleconverter can be put on the MPE 65 mm; with the 1.4x I can achieve up to about 7:1 magnification with some loss in resolution. All those lenses are strictly manual focus lenses. The camera has the extra battery pack, which also adds the high format shutter release. For macrophotography, however, I use almost exclusively an original Canon shutter release; a cheap knock-off shutter release off ebay broke within days.

I like to work with flashes for their consistent color temperature and short exposure times. I use a Canon MT-24EX with Garry Fong puffer pop-up flash diffuser attached to soften the light. A Canon 580 EXII with diffuser cube is the second flash I use. Flashes are rarely mounted on the camera shoe. Canon offers only a 2" TTL flash cable, which is rather short. I considered various remote trigger options (infra red, radio controllers, slaves), but prefer old fashioned cables; FlashZebra offers third party flash cables, including custom work. To modify the light, I use simple white card boards, which I may cut to shape for particular shots. A 5in1 Litedisc containing both reflector as well diffuser is regularly employed.

For support I use Gitzo carbon fiber tripods with a Linhof Profi II and an Arca 1B ball head, both fitted with Arca quick releases. Both have traveled the world, have taken a lot of abuse, and still work very well. I have both a manual Adorama x/z focusing stage, as well as the Cognisys motorized focusing rail fitted with Arca quick release clamp and a ReallyRightStuff long support rail to permit movement in the x-axis for framing. Additionally, I have two Wimberly PP100 Plamps, flexible arms that can alternatively be fitted with a clamp to hold a plant or a reflector, or with a home made flash-shoe to hold the heads of the twin macro flash. I attach the Plamps anywhere: tripod, focusing stage, kitchen furniture.

Stereomicroscope

I am fortunate enough to have a cashmere set-up, consisting of a Zeiss Discovery V20 stereomicroscope (Fig. 11) with a couple of planapochromatic lenses (0.63×, 1.5×). 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 or a dedicated microscope camera (Zeiss Axiocam HRc) is tethered to the computer through which I release the shutter. I avoid touching the optical set-up and do not introduce unwanted vibrations.

Images are taken with the above digital SLR mounted on the camera port of the trinocular head and the twin macro flash or with halogen cold light sources.

For each shot, 13–150 images were taken, changing focus by tiny, constant increments. I do it manually, or with a motorized focus stepper. The CR2 RAW files were run through HeliconFocus 5.1 or Zerene Stacker 1.04, the process taking about 3–15 minutes each. Then the resulting z-stacked image was minimally cleaned up in Photoshop.✾

Acknowledgements

Bob Lauri read the manuscript and made several helpful suggestions.

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Dr. Daniel L. Geiger is a curator of molluscs at the Santa Barbara Museum of Natural History, where he is also in charge of the electron microscopy facility. He has over 30 years of photography experience on a wide range of equipment and in many settings. He has written a book chapter on scientific photography, and sells his images also through a professional agency. Images have appeared in e.g. Nature, many textbooks, and exhibits (e.g., National Aquarium, Baltimore; Los Angeles County Museum of Natural History). He is growing species orchids in two terraria and a greenhouse, specializing in *Oberonia*. He is a "Visiting Research Scholar" at the Huntington Botanical Gardens, San Marino, California, member of the Orchid Society of Santa Barbara, a board member of the Southern California Orchid Species Society, and regularly gives talks for orchid societies and events.

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