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Taking seed images on a budget

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Why is it important to have good quality seed images?

Many analysts have their own 'real' seed reference material. However, in today's world of digital images and easy sharing of information, a digital reference library of seeds that is readily accessible and can be shared is an important additional resource (Fig.1). Also, some species may not be readily available for collection, and it is also easier to e-mail a good quality image for identification purposes than the actual sample. In the past the cost of specialist equipment may also have been prohibitive for many smaller laboratories.

Good quality seed images can now be taken relatively cheaply and easily with the camera equipment and software freely available. As a photographer on contract I have had the best of both worlds, using a high quality microscope / lens system for my contract work and my own, less expensive camera set-up at home.

This article aims to show how to achieve good quality images using a standard digital SLR, a lens or two and some associated equipment such as flash units and stepping rings for under USD 4360. In addition, details for image manipulation software to tidy the background, add a scale and the copyright are provided.

All the images in this article are the copyright of Jo Jones and have been taken with my own home based Nikon camera system as detailed in Table 1. My system cannot compete with the expensive microscope based image systems to provide images of very small seeds or very high magnification for extreme detail, but I hope to provide you with information and ideas about what is possible with a more affordable set up. If you would like any more information about this article or how I take images, feel free to contact me on my e-mail at jojimages@littleloon.ca.

Basic equipment

To obtain good images, all that is needed is a digital camera with the option to change lenses and a good quality lens, ideally a macro lens. Macro lenses are specialised lens that allow up to life-size reproduction of the object (ratio of 1:1, or x1 magnification) with excellent image clarity, but because they generally have more glass elements in the lens construction they are usually more expensive.

If you can afford a more expensive camera, such as the Nikon D500, you will have more flexibility and control over your images, and depending upon how you intend to use your images the number of megapixels (mpx) can be a factor. The higher the pixel count the bigger your final image can be enlarged, but if you are only using the images for the web and publications you do not really need an expensive camera. Any camera capable of allowing enlargements of 24 x 36 inches is sufficient for poster publications, without significant loss of image quality.

Camera movement equals poor resolution resulting in less sharp images. Some form of stable platform, such as a tripod or copy stand, is therefore critically important, and extra lighting will also be needed, preferably flash, although the fibre optic lighting that many labs use for their microscopes may be sufficient. An added extra is a remote release for the shutter if you are not using a PC for control. This will eliminate any movement of the camera when the shutter is pressed by hand.

Secure your seed onto some tape (or similar) before you take any frames; this will stop it moving. Any slight movement of the seed when focusing is exaggerated at higher magnification. I use some Scotch tape on a small piece of glass which is slightly raised above a white background, or a black background for seeds with overlapping long fine hairs. A raised seed allows the flash to pass through the glass eliminating any unwanted shadows. I've found that the seed is easily removed undamaged. The background is then edited out using Adobe PhotoShop.

How to achieve an image with the whole seed in focus

If you are taking a picture of anything and want the background in focus then the depth of field is very important. Depth of field is the term used to describe just how much of the foreground, subject and background is in focus. For example, in a portrait where you wish to isolate the subject from the background, a shallow depth of field is preferred, compared to a landscape where you wish to record much of the detail. This is achieved by closing the aperture of the lens. In a typical lens, the aperture is adjusted by the aperture ring, marked in f-numbers or f-stops, which is the ratio between the focal length of the lens and the aperture. Thus, a typical 50mm lens may have apertures or f-stops ranging from f1.4 (open) to f16 or f22 (closed). A small f-number means a large aperture, allowing more light onto the camera sensor through the lens, but also only a shallow depth of field. A large number means a small aperture, allowing less light onto the camera sensor, but a greater depth of field, so that more of the image will be in focus.

In digital cameras, the sensor replaces the photographic film of older cameras. It is where the light that makes up the image is 'captured' before being 'transferred' as a digital signal to the memory card inserted into the camera.

However, in the case of magnified images of small objects such as seeds, it may perhaps be impossible to achieve a satisfactory depth of field, i.e. with the entire seed in sharp focus, simply by using a small aperture (except for very thin, flat seeds).

FIGURE 1. Dragon bean seed (*Phaseolus vulgaris*) photographed at a magnification of x2. The background, copyright and scale were added using Adobe Photoshop.

The solution is to take a sequence of individual photographs, or frames, but with each frame focused on a slightly different level of the seed. Thus, in each frame a slightly different area of the seed surface will be in sharp focus from frame to frame. In this way, over the whole sequence of frames the entire surface of the seed can be captured in sharp detail (Figure 2).

FIGURE 2. Nine frames (individual photographs) of Nyjer seed (*Guizotia abyssinica*), each with a different focal point. The final image used 154 frames.

Using software, the frames of the sequence are then merged, one on top of another, to create a final composite image with everything in focus (Figure 3). This process is called ‘stacking’. It is analogous to a panoramic photograph, in which the final picture is composed of several frames which overlap, and are ‘stitched’ together along the horizontal axis. For a seed image, the frames are then ‘stacked’ together, one on top of another, in the vertical axis. The overlap in the areas of sharp focus from one frame to the next ensures that the images can be put together seamlessly.

FIGURE 3. Final image of Nyjer seeds, stacked from 154 overlapping frames with focal points set 5µm apart.

The one essential luxury for my home system is the StackShot controller (Figure 4). This automatic system uses a motor to drive a macro focusing rail to which is attached the camera and lens. When I use this controller to take a sequence of frames, as one option I can set the first and last point of focus of the sequence, and then the distance that the rail will move for each frame between these two points. I thus know that the frames are focused, for example, 10 micrometres apart (10µm), taking the guesswork out of using manual focus. I then know that all the frames will overlap, allowing me to stack the image without any unfocused areas.

FIGURE 4. My arrangement for the StackShot and Controller. I also use a Novoflex macrofocusing rail to facilitate movement along an axis at 90° to the StackShot. For easy attachment of StackShot to the Novoflex rail and the camera to the StackShot I use quick release platforms and plates from Novoflex and Wimberley. The Novoflex rail is attached to a tripod using a ballhead, again with a quick release plate.

Software

There are now many choices of software. These are widely available and prices vary. I routinely use Adobe Photoshop CS6 as my image editing software of choice, but again there is a good selection available at competitive prices. Adobe Photoshop CS6 has now been replaced by Adobe Photoshop CC which is available on subscription from Adobe. However, the software must be capable of stitching multiple images together, either as a panoramic (also useful for seedlings images) or more importantly one on top of another as a stacked image for seeds.

To put my frames all together I use Zerene Stacker software. It will also interface with the StackShot controller so I can control the entire process by computer. Using Nikon's Control Pro software (purchased separately; Canon have an equivalent software package that is supplied with some of their cameras) I can connect my camera to the computer and use the monitor to judge focusing instead of using the viewfinder. If the camera has Live View, the image can also be seen directly on the computer monitor. Zerene Stacker can manage a very large number of frames to produce an overall composite image, and it also has some image editing functions (although I prefer to use Adobe). I export the file as a TIFF image to Adobe Photoshop for final editing, such as changing the background and adding a scale and copyright. There are now a number of software packages that compete with Adobe for functionality at various prices. Adobe

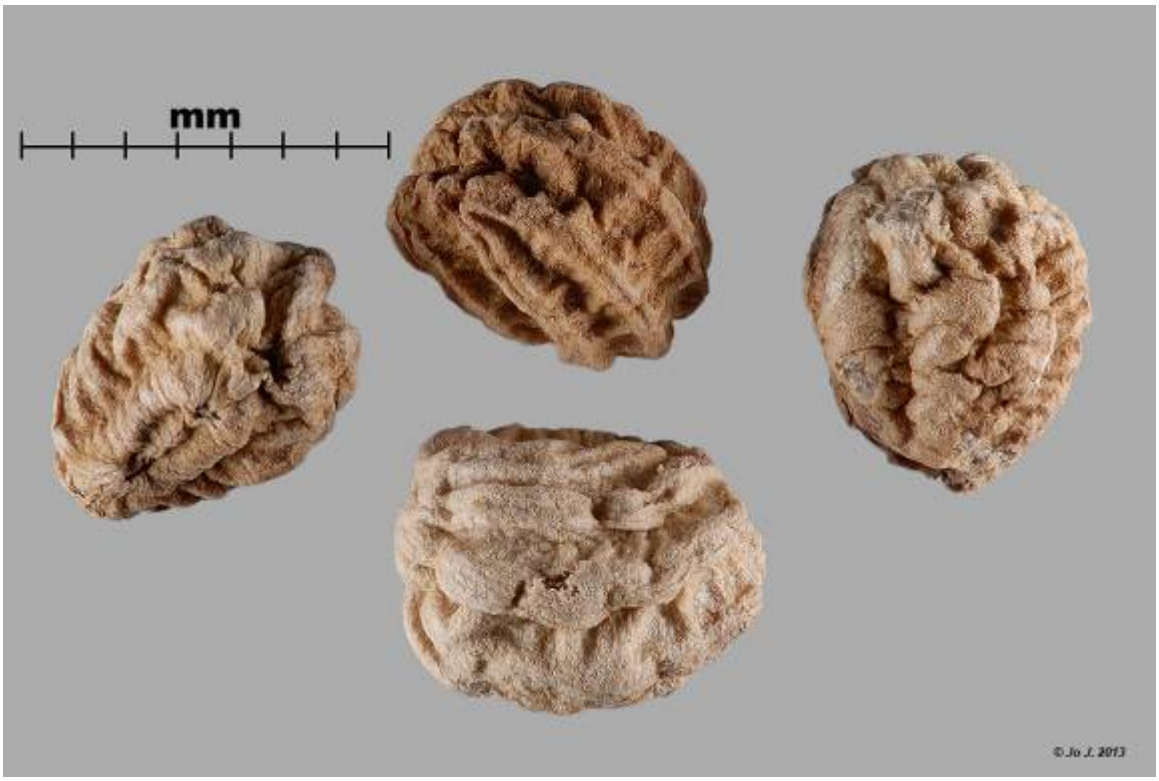
CC can be tailored to fit your needs, so the full version of Photoshop may not be needed. There are also other ‘stacking’ software packages and maybe some ‘freeware’ available, but beware of infringing any licences

Lighting

One downside to using a smaller aperture is that more light will be needed to obtain a correctly exposed frame, and also to actually see the seed to focus on (I use a desk lamp which I switch off when I take the frame). The use of supplementary lighting such as flash is necessary. Many laboratories use fibre optic lights for their microscopes, and these can also be used. However, you may get a ‘colour cast’ on your image. This can be corrected using software, but it is easier to correct before taking any photographs by ensuring that the white balance control of the camera is set to auto. This will normally compensate for a variety of different lighting situations. More recently when I need to emphasize a specific feature, I use two LED lights (Aputure Amaran AL-M9), to aid focusing and as supplementary lights for the flash system. These cost CAD 59 each and recharge using a USB connection.

Lighting can in fact be difficult to manage, and you will probably need to diffuse the light to avoid harsh shadows that can either hide diagnostic features or imply that the seed has features that are an artifact of how the image was taken. There is a wide selection of flash units available. A ring flash (centred around the lens barrel) is one solution, but I have found that lighting from both sides with two or more flash units and level with the subject gives the best lighting effect, although it does depend upon the seed (Figure 5). Nikon have a wireless macro flash system that allows you to either use the system as a ring flash or position the flash units where required (R1C1 kit). Their system also has a panel that fits over the front of each flash unit to diffuse the light. If you do not have this, a much cheaper alternative, and one that works well, is to use either a single tissue or a strip of Scotch tape over the front of the flash. It does not damage the unit and is easily removed. Wireless flash is nice, no cables to fall over, but the camera must be capable of controlling the flash when it is not physically attached to the camera. Canon produces a similar macro flash unit arrangement but their system is not wireless and the controller fits onto the hot shoe of the camera.

FIGURE 5. Nasturtium seed (*Tropaeolum majus*) from packet seed ready to grow in the garden showing varying surface features of the seed highlighted by the angle of the flash light. The final image was composed from 57 frames; each frame was 100 µm apart with significant overlap to ensure that the final image was all in focus. Two flash units were used, positioned at either side; the light was diffused to avoid harsh shadows.



Magnification up to x10 without using a microscope

To illustrate any diagnostic features or surface texture, magnification can be increased relatively simply and cheaply by mounting a lens back-to-front either directly onto the camera body or onto another lens, by using reversing rings. These screw into the filter thread in the front of the lens, and are widely available in various sizes for a few dollars (or pounds or euros) in camera stores or online. The reversed lens does not need to be the same make as the camera, as it is not being attached directly to the camera body. Reversing works because it increases the camera sensor to lens distance compared to the subject to lens distance (a standard lens is designed to give ideal results when the lens to camera sensor distance is less than the subject to lens distance). However, the lens must have an aperture ring; for example Nikon's series of G lenses will not work. An old manual-focus lens is ideal, and these can be found second-hand from some camera stores, as these days few people want to use manual-focus lenses.

If reversing rings with the correct filter thread are not available, temporary but workable attachments may offer a solution. For example, taking images of seeds for this article I temporarily joined two lenses with different filter threads. My 105 mm lens has a 52 mm filter

thread, my 20 mm lens one of 62 mm; using several rings and duct tape I joined these together without any loss of image quality (Figure 6).

FIGURE 6. A 20 mm lens reversed onto a 105 mm macro lens resulting in approximately a magnification of x5 (top figure) and a Nikon BR-2A adapter attached to the camera body to reverse a 20 mm lens using reversing rings to attach the lens for a magnification of x3.4 (lower figure). The silver is a thin strip of duct tape used to join the rings together to keep a tight, light-free seal.

An alternative method to increase magnification but will increase the cost is to use a x2 teleconverter. This doubles ‘magnification’ but also affects aperture, although image quality is not greatly affected. Teleconverters are also available as x1.4 and x1.7 and are available from the major camera manufacturers and independent makers such as Kenko.

Reversing my ‘old’ 20mm lens directly onto the camera body using a Nikon BR2A adapter increased magnification to x3.4. Adding a x2 teleconverter to the front of this arrangement increased it to x6.8 (Figure 7).

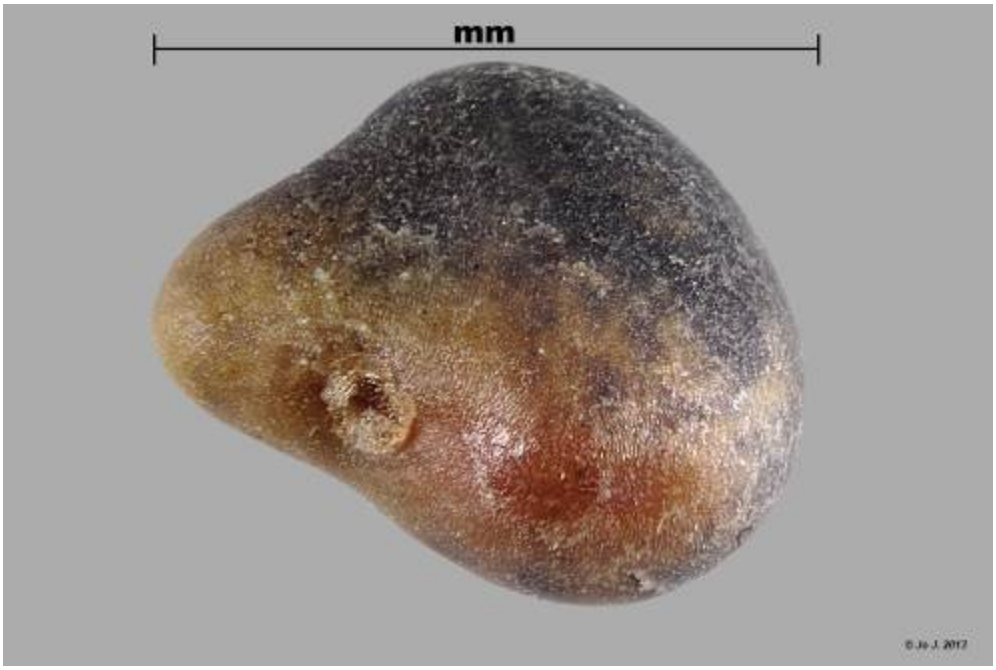
FIGURE 7. Poppy seed (*Papaver sp*.) taken with a reversed 20 mm lens and a x2 teleconverter (Magnification of x6.8).

The 105 mm macro lens alone produces a life size image (Figure 8). Reversing the 20 mm lens to the front of this lens increased the magnification to a ratio of 5:1 (Figure 9). Adding a x2 teleconverter will increase this further to 10:1 (Figures 10 and 11).

FIGURE 8. Home -saved dragon beans (*Phaseolus vulgaris*) photographed using a 105 mm macro lens at a magnification of x1.

FIGURE 9. The surface features of this onion seed (*Allium cepa*) can be clearly seen at a magnification of x5. The image was composed from 100 individual frames; the distance between each frame was 15 µm.

FIGURE 10. Clover (*Trifolium sp.*) from bird seed taken at a magnification of x10. A total of 107 frames were taken and stacked together, frames were 5 µm apart.



A consequence of the higher magnification is that the light entering the camera is very low, but a flash system can compensate for this. The reversed lens must be wide open, i.e. the aperture must be at as low a number as possible.

Fine details such as that identified in Figure 11 can aid in seed identification where the surface features can be easily seen. This image could be compared to seeds of other grasses which could be prohibited or noxious species.

FIGURE 11. A grass seed from a lawn seed mix showing the features of the seed at the rachilla, x10.

Another relatively cost effective way to increase magnification is to use a close-up lens which screws to the front of your lens as a filter. These are available at most camera stores and range from +1 to +4.

Some other tips

I use a calibrated monitor to ensure that the seed colours are true to life.

I also take my individual frames as JPEG images, using the largest JPEG format available on my camera, with the least compression. This is principally because RAW files are so large that handling a large number can cause the computer to 'hang'. If you convert the stacked image as a TIFF for further work, no loss of image quality is evident. I have found that the colour difference that can sometimes be seen between RAW and JPEG images is not usually an issue when photographing seeds.

Remember to take as high a resolution image as possible, such as 300 ppi. It is easier to crop an image, and maintain the fine detail, than to try to add extra pixels in the software package.

Table 1. Approximate costs for a Nikon based camera and flash system, for other manufacturers see the websites listed in Table 2.

Equipment	Guide price (USD)
Camera, Nikon D500 (21 Megapixels)	1400
105mm f2.8 macro lens	900
*R1C1 flash kit (two versions available)	460 or 700
Tripod and head	100 to 300
**StackShot controller, include QR platform, remote release	800
QR clamp and plate for camera body	150
***Zerene Stacker (full licence)	300
Additional second hand lens and x2 converter	100
Reversing rings	50
Image editing software	100 to 310
TOTAL USD	**4360 - 5010**

Prices do not include any taxes or shipping and are for guide purposes *only. Most software is now available on monthly subscription, price quoted is for single user without subscription.*

*Cheaper flash units are available; ensure that the unit is compatible with your camera system.

**The StackShot can be customised according to your requirements, therefore the price is variable.

***This is for a full licence for Zerene Stacker. A licence for a not-for-profit organisation is USD 90 and for students USD 40.

Table 2. Websites and useful contacts

Product	Company	Website (www.)
Adobe Photoshop (software)	Adobe	Adobe.com
***Zerene Stacker (software)**	Zerene Systems	zerenesystems.com
Nikon camera systems and software	Nikon	Nikon.com
Canon camera systems	Canon	Canon.com
***StackShot controller**	Cognisys	cognisys-inc
Macrofocusing rail	Novoflex	novoflex.com
***Quick release platform and plates**	Wimberley	tripodhead.com
***Ballhead for tripod**	Acratech	acratech.net
****Reversing rings, filters etc**	Speedgraphics	speedgraphic.co.uk
*****Reversing rings, filters etc**	The Camera Store	thecamerastore.com
***Reversing rings, filters etc**	B&H	bhphotovideo.com

* Website prices quoted in USD. **Website prices quoted in GBP. ***Website prices quoted in CAD.

Most companies will ship Worldwide.

Websites and contact details for Jo Jones

Gallery: www.pbase.com/joj

http: //joj.images.tripod.com

email: jojimages@littleloon.ca

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26 April 2020. Jo Jones