

Photographing The Fern Gametophyte Developmental Series

– The First Attempt

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For years I have wished to photograph a series of fern gametophytes, showing development from the single cell to the mature gametophyte with emerging sporophyte leaf. I have recently begun this project in earnest and have my first fairly good photograph to show.

The difficulty in years past has always been that in photography at such small size the depth of field of the photograph is very narrow indeed. The gametophyte therefore appears as a fuzzy blob with a stripe of focussed cells across the middle (Fig. 1).



Fig. 1. Fern gametophyte single photograph showing the problem of very narrow depth of field.

However, with the advent of digital photography, a new technique called 'focus stacking' has been invented, which brings great opportunities for photography at this scale.

The idea of focus stacking is that the fern gametophyte is photographed repeatedly, and that between shots, it is moved ever-so-slightly towards the camera. As the gametophyte moves, the part of it that appears in focus in the photograph changes. Many single shots can be taken, and ultimately somewhere in this large collection of shots, there will be a small focussed picture of every part of the fern gametophyte.

The tricky part is to take all of these focussed fragments and put them together like a jigsaw to form one single photograph, in which the whole gametophyte is perfectly focussed. This is very difficult, but there is now software that will do a good job, if the slices (individual photographs) are of good enough quality.

I have attempted to use focus stacking to take a good photograph of a fern gametophyte. As the subject is so small (approx 2mm across), the focus stacking steps also have to be very small indeed (<0.02mm). Specifically, this is because the Canon lens that I am using has a very high magnification (x5) but a depth of field at this magnification of about 0.048mm at f/2.8. There is commercial equipment

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available to move the specimen in these tiny steps, but it is very expensive and so I built my own, adapting instructions from a rough guide that was shown on the internet, and with the general idea recommended to me by Professor Jim Haseloff of Cambridge University.

The setup involved stripping down a Plustek OpticPro U12 flatbed scanner and removing all of the driver boards. I then wired in a Raspberry Pi computer via a breadboard, and linked the Raspberry Pi computer to a laptop, from which I programmed the computer. The whole set up is shown in Fig. 2. Further detail of the wiring is shown in Fig. 3 and the wiring map including the chipset diagram for chipset ULN2003 that I used is shown in Fig. 4.



Fig. 2. The focus stacking setup.



Fig. 3. Connection of the Raspberry Pi to the breadboard, and on to the flatbed scanner.

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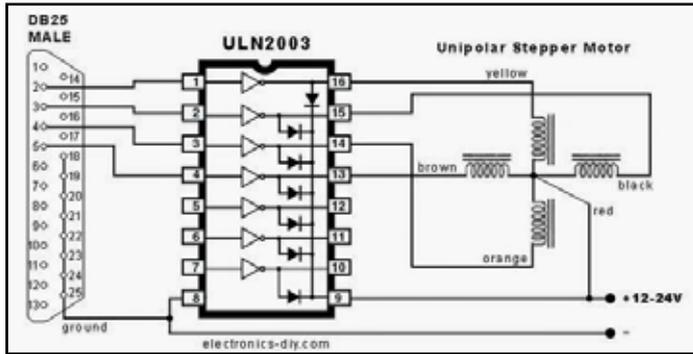


Fig. 4. Manufacturer's chipset wiring diagram for ULN2003 chip. (This diagram is so widely distributed online that I have been unable to find out who the original author was, for attribution.)

Working out how to control the stepper motor in the flatbed scanner was quite a complicated business but it was managed by experimentation and a computer programme was written in the Python language to control the movement of the motor in the correct way to allow the photographs to be taken.

Below is the code of the programme that was used. I include this to help others who may be trying to replicate my work. The code was later modified to enable the space bar to be pressed each time a step advance was needed, which enables much finer control of the timing.

```
#!/usr/bin/env python
import RPi.GPIO as GPIO
import time

GPIO.setmode(GPIO.BCM)

#This assigns the pins of the raspberry pie to variables called "pin".
pin0 = 18
pin1 = 23
pin2 = 24
pin3 = 17
#
GPIO.setup(pin0, GPIO.OUT)
GPIO.setup(pin1, GPIO.OUT)
GPIO.setup(pin2, GPIO.OUT)
GPIO.setup(pin3, GPIO.OUT)

#Zero all of the pins between tests.
def zero_pins():
    time.sleep(0.1)
    GPIO.output(pin0, 0)
    GPIO.output(pin1, 0)
    GPIO.output(pin2, 0)
    GPIO.output(pin3, 0)

#This runs the test.
def test():
    count = 1
    while (count < 9):
        print 'The count is:', count
        zero_pins()
        GPIO.output(pin0, 1)
        time.sleep(1)
        GPIO.output(pin1, 1)
        zero_pins()
        time.sleep(1)

        GPIO.output(pin1, 1)
        GPIO.output(pin2, 1)
        zero_pins()
        time.sleep(1)

        GPIO.output(pin2, 1)
        GPIO.output(pin3, 1)
        zero_pins()
        time.sleep(1)

        GPIO.output(pin3, 1)
        GPIO.output(pin0, 1)
        zero_pins()
        time.sleep(1)
    count = count + 1
test()
```

Once the stepper motor of the scanner was fully under control, the camera was set up so as to be stable and close to the subject. The full setup is shown in Fig. 5. As can be seen, the camera lens sits very close indeed to the subject (this can be seen with a much larger *Cymbalaria muralis* leaf subject in Fig. 6). The photography subject is attached to the top of the scanner arm using bluetac. There are two diffused Yongnuo YN560-IV Speedlite flashes very close to the subject that are fired using a remote control device on the top of the camera body. The optical equipment list is as follows: Canon DSLR camera 5d MkII, Canon MP-E 5x macro lens, and two extension tubes Canon EF25II and EF12II. This lens and extension tube combination produces approximately x8 magnification on to a full frame (35mm) sensor in the camera body. The camera shutter was fired using the Canon EOS Utility software on the laptop computer, via a USB wire. The Raspberry Pi was also controlled from the laptop, so that alternately the shutter could be fired and the scanner arm advanced.



Fig. 5. Complete setup including camera.



Fig. 6. Close up view of a larger subject in front of the lens, showing the scale of the photography involved.

Growing a suitable specimen proved tricky as I live in Cambridge and a sunny windowsill here is really too hot for a fern gametophyte. However, a sunny windowsill

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in December proved to be ideal and I grew a good crop of *Asplenium scolopendrium* (Hart's tongue fern) gametophytes. The plants were grown from spores harvested in my garden, in pots on 'Jack's Magic compost.' The compost was sterilised with boiling water and cooled in the fridge. The spores were sown, and the whole bagged, and left in an airing cupboard with a large window, for approximately three months. A single specimen from the germinated population was used, and as luck was on my side that day, I took quite a good shot on my first attempt.

Using my setup, the process of firing the shutter and moving the sample was carried out forty times to produce forty slices (or single photographs).

The forty individual slices or photographs were then amalgamated using Helicon Focus software, which is a commercial software package designed for focus stacking. The amalgamation worked quite well and the resulting photograph is shown in Fig. 7.

I was pleased with this image as it is fairly sharp across the whole subject. However, it is also quite blown out with specular highlights and a slight lack of sharpness

across the entire subject. I have been in touch with some macrophotography experts at the website <http://www.photomacrography.net/> and they have given me advice on how I could improve my images using microscope objectives instead of the Canon MP-E lens. I have been looking into this option and hope to apply for a grant to buy better equipment and to continue on my project to take a developmental series of photographs showing a fern gametophyte as it progresses from the single cell to the mature gametophyte with emerging sporophyte leaf. I'm still very pleased with my first image though, and I hope that you enjoy it as much as I have.

I would like to acknowledge the contribution of my husband Tim Deegan, who helped with the computing and engineering setup of this system, Malcolm Wilkins of the University of Glasgow for introducing me to the study of fern gametophyte development, and Jim Haseloff of the University of Cambridge for suggesting the use of a flatbed scanner. I would also like to thank my parents, Irene and Ewan Clark, for providing the many years of Christmas and birthday money that funded this project. □



Fig. 7. The focus stacked image.