

Application of Newton's Zero Order Caustic for Analysis and Measurement: Part II – Fluorescence

Antonio A. Garcia^{1*}, Luis Nuñez², Carissa Henricksen¹, Vladimiro Mujica³

¹*School of Biological and Health Systems Eng., Arizona State University, Tempe, AZ, USA*

²*Center for Green Manufacturing, University of Alabama, Tuscaloosa, AL, USA*

³*Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ, USA*

ABSTRACT

An oblate spheroid-shaped liquid solution acts as a lens through the phenomenon of Newton's zero order caustic resulting in the focusing of a low power source into high intensity light within the sample to detect and measure fluorescence. Microwatt LED sources can be used to measure fluorescence in two ways, depending on the properties of the solute. For a weakly fluorescing solute, emitted light can be detected as a beam emanating from the spheroid sample along the path of illumination. For strongly fluorescing solutes, emitted light can be detected radiating from the surface orthogonal to the excitation source. Three fluorescent molecules (tartrazine, SYBR Green I, and BBT anion) were studied using photodiode excitation sources of 430 and 470 nm maximum wavelengths respectively. Image capture for strongly fluorescent molecules was performed with a digital phone camera, followed by image analysis in order to calibrate light detected as a function of fluorescent molecule or complex concentration.

Keywords: Newton's zero order caustic, fluorescence, SYBR Green I, tartrazine, ray tracing

1. INTRODUCTION

Fluorescence measurement is a versatile and widely used method in diagnostics, molecular and cellular biology research, environmental studies, and chemical analyses. Advances in nanomaterials, highly sensitive fluorescent detection strategies, as well as probes with a wide array of emission wavelengths, continue to expand its use, especially when specific detection and measurement is desired at very low concentrations – even down to a single molecule. In the first part of this 3 part series, Newton's zero order caustic was shown to be an optical system that can generate high intensity light within the sample being detected, without the use of high power light sources, lasers or lenses. In fluorescence, a relatively high intensity of light at the required excitation band is usually preferred, as long as photobleaching can be minimized, in order to maximize the detection range and sensitivity. Emission of fluorescent light at low fluorophore concentration is given by the equation:

36

$$I_f = 2.3F \Phi_f I_o dC \quad (1)$$

37 where Φ_f is the quantum efficiency; I_o is the intensity of the excitation light; ε is the molar
 38 absorption coefficient of the fluorescent molecule; l is the path length, and C is the molar
 39 concentration of the fluorescent molecule (or limiting species if a probe fluoresces in the
 40 presence of a target molecule). For example, the DNA detection molecule SYBR Green I is
 41 considered to have a molar absorption coefficient of 50,000-70,000, quantum efficiency of
 42 0.8, and at a dilution of 1:5,000 from the standard 10,000x stock, a concentration of 3.9
 43 micromoles per liter. Generally, quantitative fluorimetry is done using instrument-specific
 44 factors rather than explicitly calculating quantum efficiency, absorptivity and path length. The
 45 emission spectrum is normally of longer wavelength than that of the excitation band, and if
 46 this difference, which is known as the Stokes shift, is sufficiently separated, detection of the
 47 emitted light can be performed, although optical filters are frequently employed as well.

48 Fluorescence detection can be performed using a variety of geometries, filters, and light
 49 collection methods, depending upon the particular application. One method that uses
 50 fluorescence from a spherical liquid sample in air is FACS (fluorescence-activated cell
 51 sorting) whereby cells suspending in small drops are irradiated with a laser, sorted and
 52 collected, typically based on the fluorescent characteristics of a cell labeled with fluorophore-
 53 conjugated antibodies. However, the authors are unaware of a method or device whereby
 54 excitation light is intensified within a liquid sample for detection and measurement of the
 55 fluorescing species concentration.

56 Fluorescence microscopy and spectroscopy rely on excitation sources in the range of 10-
 57 100 W, while a single, low cost LED is typically much less powerful using 50-100 milliwatts of
 58 electrical energy and providing less than a milliwatt of light energy in a particular direction.
 59 Thus, being able to generate sufficient intensity of excitation light within a sample using low
 60 power LED sources would be a very useful way to lower power needs in fluorescence
 61 measurements.

62 In part I, it was shown that an oblate spheroid can generate a cusp of revolution and an axial
 63 spike caustic within the spheroid due to the refraction of light without any internal reflections.
 64 The description of light rays incident on a drop of water with refraction but without reflection
 65 is termed Newton's zero order caustic in the literature [1,2]. For oblate spheroids where
 66 incident light is aimed at the major axis, not only are light intensities in the forward scattering
 67 direction significantly higher than the incident light intensities, but for a wide range of aspect
 68 ratios the highest intensity light can be focused in the center of the sample [1]. Due to the
 69 high forward illumination intensity in this system, it would then be reasonable to expect that
 70 fluorescence could be generated within the sample, but it is unclear how the excitation light
 71 and sample shape can be best employed to simplify detection while maintaining high
 72 sensitivity and linear calibration at low fluorophore concentration.

73 This paper explores the use of Newton's zero order caustic using experimental results for
 74 fluorescent solutions shaped into spheroids and measured via a simple optical platform.
 75 Two-dimensional ray tracing is used to qualitatively illustrate where high intensity excitation
 76 light occurs using different spheroid shapes, which can be created by lowering the surface
 77 tension of the sample. Relevant scientific literature on light scattering and rainbows is also
 78 used to help link 2-D ray tracing with experimental results.

79

80

81 2. MATERIAL AND METHODS

83 2.1. Sample Stage

84
85 The sample stage consisted of a PTFE coated slide with 30 wells of 3 mm diameter (Tekdon
86 Inc., Myakka City, FL). Two wells along the center line of the slide were employed to pin
87 drops of 160 microliters of the liquid sample, forming a flattened spheroid shape without the
88 need of a sample container. Each multi-well slide can measure 5 different samples and
89 each position can be used for multiple tests of the same sample solution. At room
90 temperature, evaporation is very slow since the aqueous drop is pinned and held in an
91 oblate spheroid shape rather than allowed to spread into a thin film. Both the lower surface
92 to volume ratio of the spheroid and the stagnant film of air on the lower half of the sample
93 contributed to low evaporation rates such that the sample volume changed only by 7% after
94 15 minutes, which for the purposes of fluorescent measurements for highly fluorescent
95 molecules, introduces negligible variations in the data. The oblate spheroid shape of the
96 samples were verified using ImageJ (<http://rsbweb.nih.gov/ij/>) and found by edge analysis
97 and measurement to have ratios of major to minor axis typically in the range of $b/a = 1.5 -$
98 2.0 , depending upon the concentration of surfactant in the drop and the relative effects of
99 pinning and surface tension that balance and keep the sample intact. Due to drop spreading
100 via gravitational effects for a larger 160 microliter drop as compared to the 120 microliter
101 drops used in Part I, the sample has a flat zone on the bottom of the drop. This shape
102 deviation is not considered to negate the assumption of a oblate spheroid used in 2-D ray
103 tracing because experimental results corroborate the model predictions. Also, for
104 measurement of fluorescence from an orthogonal position using highly fluorescing
105 molecules, the change in drop shape does not affect the ability to calibrate the system using
106 the integrated density software tool in ImageJ. Refraction, reflection, and light loss due to the
107 material properties of a container is avoided in the experimental platform since the sample is
108 a free standing drop.

109 2.2 Photodiode Device

110 Excitation light was generated using two different blue LED photodiodes (430 nm and 470
111 nm peak wavelengths) with internal micro-lens and a PBT housing (Industrial Fiber Optics,
112 Tempe, Arizona). Each photodiode was powered by a standard, constant voltage, DC circuit
113 based on the manufacturer's recommended value of 4.5 Volts at 35 mA using a 9 volt
114 battery. The spectral bandwidth of the 430 nm photodiode is wider (65 nm) than that of the
115 470 nm photodiode (25 nm). Typical optical output power levels for these photodiodes,
116 which are normally used in fiber optic communications, are 25 micro Watts for the 430 nm
117 photodiode and 75 micro Watts for the 470 nm photodiode.

118

119 2.3 Fluorescence Measurements

120 The same optical holder for the 30 well slides was used in Part I of this 3 part series.
121 However, only one photodiode was used and the acrylic body was painted flat black.
122 Measurements were conducted in a dark room, with a low wattage desk spot lamp
123 pointing away from the sample stage in order to apply the samples and record
124 images or spectra. In order to conduct the measurements for SYBR Green I and
125 BBT anion, an 80 microliter aliquot of the fluorescent molecule solution is first
126 applied to the slide followed by a second 80 microliter aliquot of DNA or alkaline
127 phosphatase. Mixing of drops using these 30 well slides has been observed to take

128 place in less than 2 seconds, and thus images can be acquired shortly after the
129 second drop is applied. Typically, the photodiode is left on during the experiments
130 for several hours since the power usage is relatively low.

131 Detection of light when studying SYBR Green I and BBT anion samples was
132 conducted using an HTC Incredible 2 Android phone camera to acquire images.
133 The ImageJ software program was used after the photo image was recorded to
134 apply a digital color filter (selecting the green filter image), and measure the
135 integrated density in a section of the spheroid sample. Background fluorescence
136 near the drop was also recorded and subtracted from the integrated density
137 measurement. The camera was kept between 4-5 cm from the sample.

138

139 **2.4 Spectrophotometry**

140 For tartrazine, a fiber optic spectrometer (model USB2000 Miniature Fiber Optic
141 Spectrometer, Ocean Optics) was used to detect the light exiting the spheroidal
142 sample. This spectrophotometer accepts incident radiation transmitted through a
143 single-strand optical fiber (400 μm fiber diameter, UV/VIS Laboratory-grade Patch
144 Cord Optical Fiber Assembly, Ocean Optics) and disperses it via a fixed grating
145 across a 2048-element linear CCD array detector. The operating software
146 (SpectraSuite, v. 2.0.109, Ocean Optics) runs on a Mac Pro laptop (Apple,
147 Cupertino, CA). An achromatic collimating lens (350-2000 nm, model 74-ACR,
148 Ocean Optics) was used to collect the incident light, which is then transmitted
149 through the optical fiber to the grating. Spectra were taken as described in the
150 experimental section of Part I of the 3 part series.

151

152 **2.5 Chemicals and Reagents**

153

154 Tartrazine exhibits a maximum light absorbance at 427 nm and fluoresces when
155 excited by a relatively broad band of UV light (280-380 nm), generating a strong
156 fluorescent light at 565 nm [3]. SYBR Green I, N',N'-dimethyl-N-[4-[(E)-(3-methyl-
157 1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-
158 1,3-diamine, (Invitrogen, Grand Island, NY) is an asymmetrical cyanine dye that
159 when bound to dsDNA absorbs blue light at a wavelength maximum at 497 nm and
160 emits green light at a wavelength maximum at 520 nm. In the absence of dsDNA,
161 SYBR Green I has a relatively weak, but detectable, fluorescence. BBT, 2'-[2'-
162 benzothiazoyl]-6'-hydroxybenzothiazole, (Promega, Madison Wisconsin) is a
163 substrate for alkaline phosphatase and fluoresces in its anionic form after enzymatic
164 reaction, producing a fluorescent species with a wavelength maximum for excitation
165 at 435 nm and an emission wavelength maximum at 575 nm.

166

167

168 **3. RESULTS AND DISCUSSION**

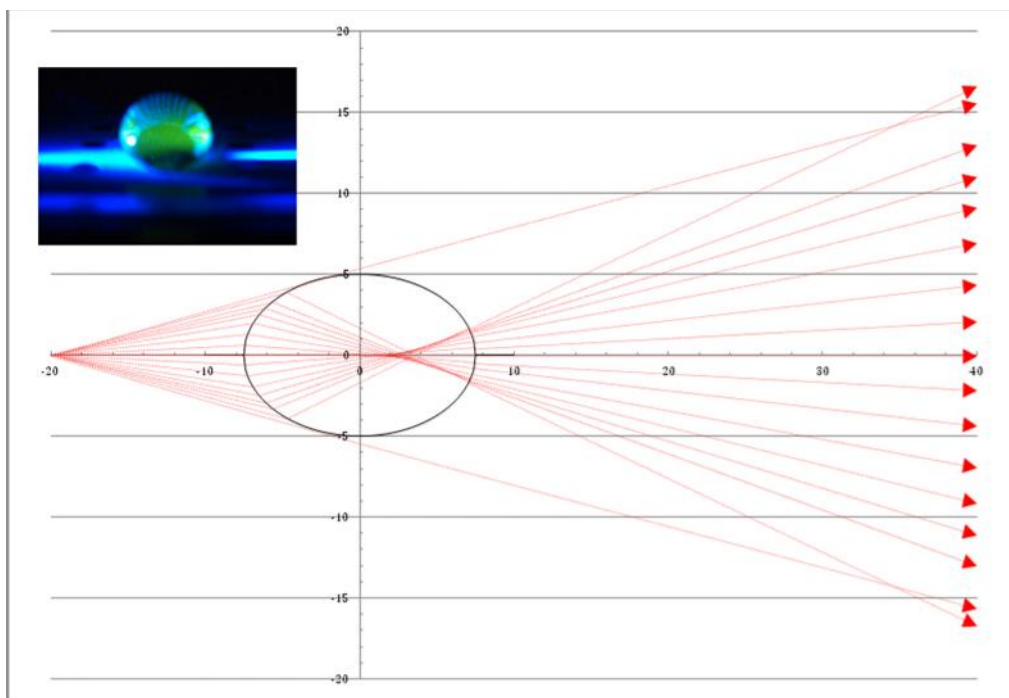
169

170

3.1 2-D Ray Tracing

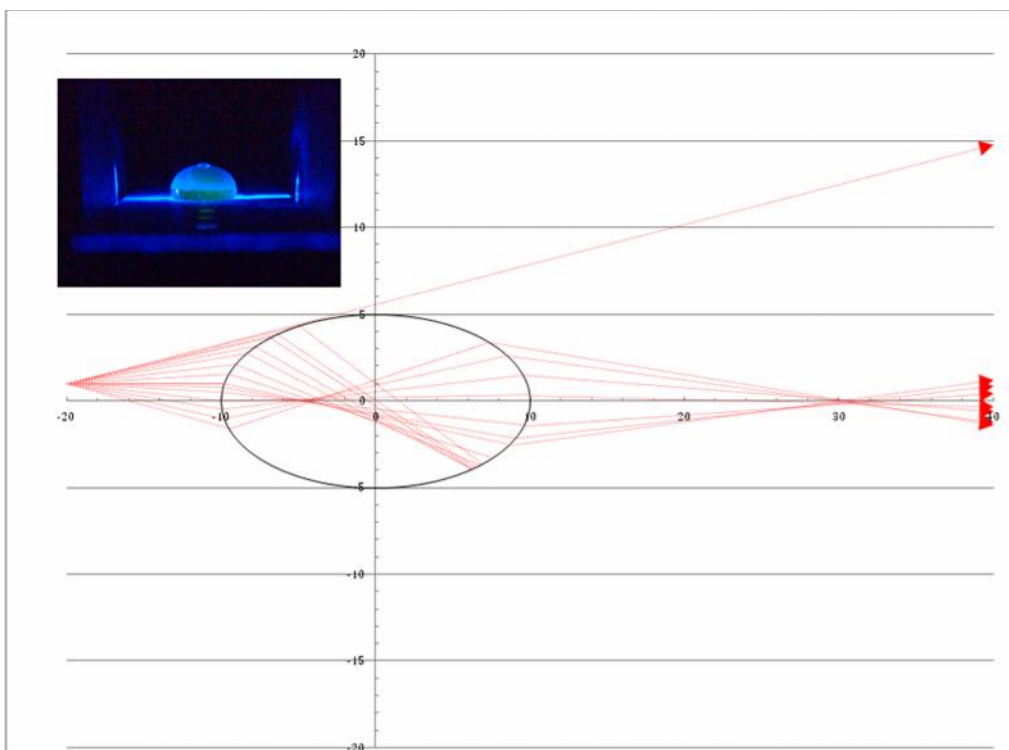
Light ray tracing analysis in the literature [1,4] indicate that light irradiating an oblate spheroid shaped sample edge-on will coalesce within its volume into Newton's zero order caustic. Through observation of different fluorescent species and solutions with and without surfactants, it became apparent that the volume and aspect ratio of the spheroid affect the fluorescent light detected since the source light used is of very low intensity and requires focusing within the sample to generate an easily detectable fluorescence intensity. Also, the position and geometry of the excitation light source with respect to the center of the drop is crucial for understanding the way light refracts within the sample. Lock [1] and Lock and McCollum [2] derived equations for the ray tracing of light with no reflections for spheres, elliptical cylinders, and spheroids. The equations from these authors are used to compute the path of light rays in 2-D in order to better interpret images and observations of fluorescence. Computed and graphed results were compared to similar geometries and source types (point or uniform) given in these articles, as well as by Kofler [5], in order to verify the detailed calculations.

Figure 1a illustrates rays for a point source placed at a distance from the center of the sample of four times the spheroid's minor axis ($x_s/a = 4$), with a ratio of major to minor axis (b/a) of 1.5. It should be noted that the bottom of the sample drop (see insert for SYBR Green I detection of dsDNA) is flatter than this idealized representation, but the difference is not significant for the purposes of this discussion since ray tracing is being used solely as a qualitative guide to indicate where the highest intensity light is found. Figure 1b shows ray tracing for a spheroid with $b/a = 2.0$, where rays exiting the drop $y < -5$ are not shown to approximate the more pronounced difficulties in justifying using an oblate spheroid as the appropriate geometry since there is an appreciable flattening of the drop, for this b/a ratio. In this Figure, the caustic focus appears to be shifted to near the part of the spheroid where light enters, which is directly attributable to the higher value of b/a and the fixed position of the light source in the experimental system used. To illustrate the significance of this effect, the insert to Figure 1b shows an experimental image for $b/a=2$ and the top half of the spheroid is seen to have a diffuse glow of blue light. Not shown is that in the room light the sample in the Figure 1b insert appears essentially like water and similar in clarity to the insert of Figure 1a. By comparison, in Figure 1c, a 2-D ray tracing of a spherical sample shows that offsetting the point source for a sphere shifts the caustic focus slightly below the plane $x = 0$. This exploration of shape and point source position illustrates that 2-D ray tracing can help interpret deviations due to sample and source geometric changes. Figure 1a and 1b also indicate that the spatial location within the sample most useful for quantitation is influenced by b/a .



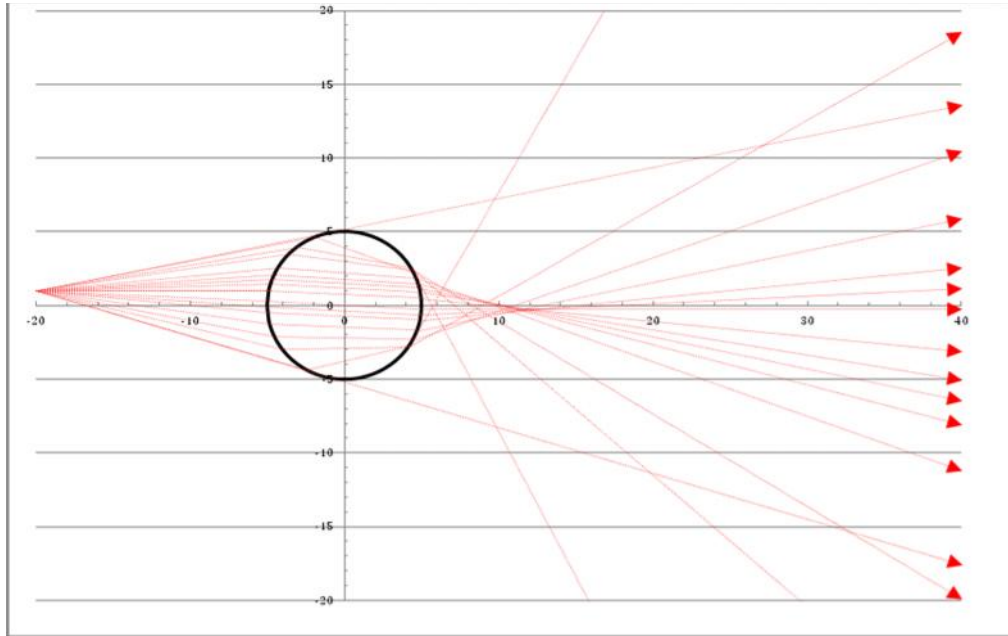
208

209 (a)



210

211 (b)



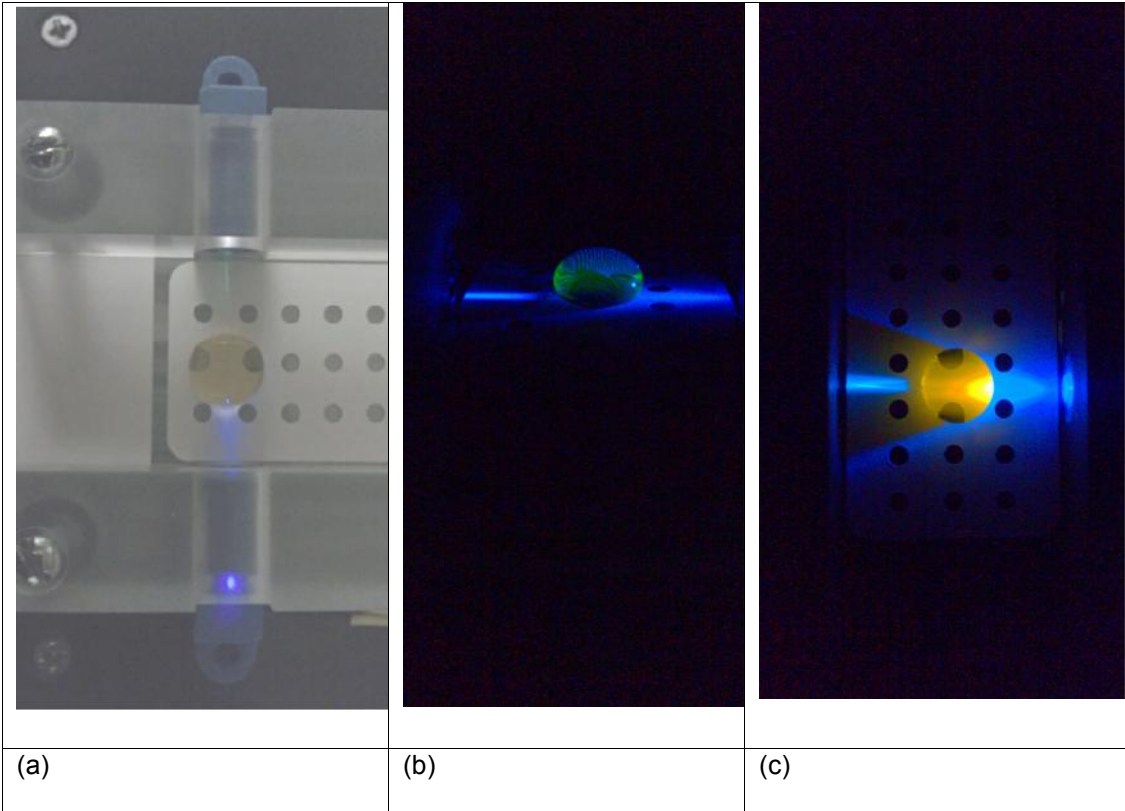
(c)

Fig. 1. 2-D Ray tracing for (a) a centered point source illuminating an oblate spheroid of $b/a=1.5$, (b) a point source offset from the centerline with respect to an oblate spheroid of $b/a=2$ with some bottom rays removed to represent effect of spreading of a liquid sample at high surfactant concentration, and (c) a point source offset from a sphere for comparison purposes. Figures 1a insert is an image of SYBR Green I with calf thymus DNA with $b/a=1.5$. Figure 1b insert is another image of SYBR Green I with calf thymus DNA with added surfactant in order to increase b/a to a value of 2.

It should be noted that 2-D ray tracing of a point source illuminating a spheroid is geometrically problematic since spheroidal symmetry does not match the spherical symmetry of a point source, making it difficult to see how Figures 1a and 1b could be directly interpreted in 3-D. Lock and McColum [2] correctly matched a point source to a cylinder of water with an elliptical cross section for their ray tracing, and Lock [1] limited his study of oblate spheroids to uniform sources. However, even in these cases, Ren and colleagues point out that interference effects of all orders of rays are not taken into account making it difficult, in the 2-D approach, to take into account scattering in all directions which can be done with a vectorial complex ray model instead [6]. Horvath et al [7] studied water drops on leaves, provided ray tracing calculations for sunlight at different angles and drops that took into account flattening at the bottom of the drop due to the leaf surface. Qualitatively, the 2-D results in Figures 1a and 1b follow similar trends in the position of the caustic focus with b/a and incident light angle shown in all of these studies, so it is assumed that as long as the 2-D ray trace is not considered to represent a symmetrical 3-D result, the shape and coordinate mismatch should not limit their use in qualitatively interpreting the experimental images.

3.2 Comparison of Fluorescent Molecules

240 Figures 2 a-c compare the way fluorescence can be observed through the use of Newton's
241 zero order caustic. In Part I, it was found, based on deviation of the absorbance calibration
242 from the Beer Lambert law, that tartrazine fluoresces at higher solution concentrations. It
243 was also shown that the fluorescence could be detected by analyzing the spectra of the light
244 exiting the sample. By comparing Figures 2a and 2c, which have high concentration (0.2
245 millimolar) of tartrazine and BBT anion respectively, beams of colored light are apparent in
246 both images. This effect is more striking in Figure 2c since the emitted light is yellow and, as
247 predicted by ray tracing in Figure 1a, there is a divergence of light exiting the sample. The
248 more intense beam of blue light emanating from the sample in Figure 2c is an interesting
249 contrast to what is seen in Figure 2a where the light corresponds to the emission spectra for
250 tartrazine. The difference between Figure 2a and 2c is attributed to both the higher molar
251 absorbance of tartrazine for the excitation light, the respective bandwidths of the excitation
252 sources, and the higher fluorescence of BBT anion. At 0.2 millimolar concentration,
253 tartrazine absorbs much of the 430 nm blue light. But the excitation of tartrazine by light of
254 less than 400 nm wavelength results in fluorescence that can be visually observed, even in
255 room light. On the other hand, BBT anion is used at low concentration and much of the 470
256 nm light concentrated along the axis of the sample is not absorbed by the sample.



257 **Fig. 2. Observations of fluorescence for tartrazine (a) , SYBR Green I with DNA (b),**
258 **and BBT anion with ALP (c), in order to compare and contrast how fluorescence is**
259 **observed and to illustrate the use of 2-D ray tracing as a guide to interpret the**
260 **features seen in (b) and (c).**

261

262 The same light source used to generate the image in Figure 2c was used to generate the
263 image in Figure 2b, which was created by combining SYBR Green I with 80 ng of calf

thymus DNA. In a typical experiment, with diluted SYBR Green I and low surfactant concentration, there is a distinct green color within the oblate spheroid, however striped lines of blue are seen at the top and some green patterns on the sides. These striped patterns are believed to be reflections of light from the Teflon coated slide and glass wells at the bottom of the slides. Another important observation made through imaging hundreds of fluorescent images of SYBR Green I [8] and BBT anion solutions is that the bottom middle of the sample is relatively dark, which is not surprising since the 2-D ray trace suggests that the spheroid receives no refracted or transmitted light in that region. When viewing BBT anion solution from the side (Figure 3), blue stripes also appear at the top of the spheroid until the concentration reaches a level where the fluorescent light is bright enough to mask the reflected excitation light.

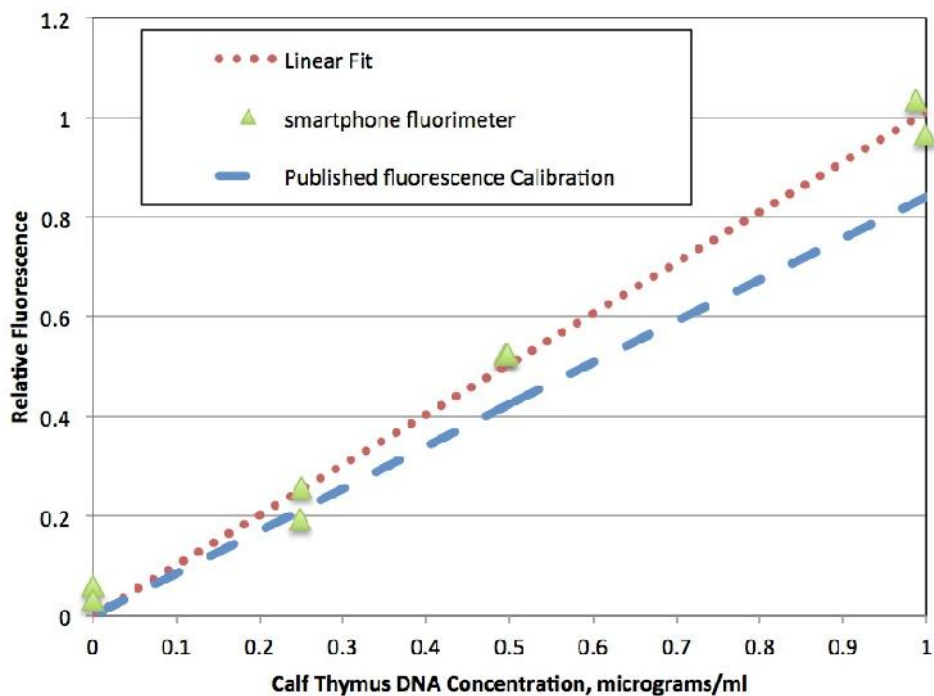
275

276 3.3 SYBR Green I

277

278 Based on the calculations by Lock, for a spheroid where $b/a = 1.5$ and using a planar source
279 of light, end on, there is a high degree of forward scattering in small oblate spheroid water
280 drops with a peak increase in intensity to nearly $I/I_0 = 1 \times 10^6$ at scattering angles between 0
281 - 2 degrees, and with I/I_0 between 100-1,000 for scattering angles between 3-30 degrees.
282 Since power is proportional to intensity, if area is kept constant, this calculation suggests a
283 sizeable increase in light intensity is possible with this experimental optical platform. Kofler
284 [5] used geometric and wave optics to predict a global intensity increase at the caustic focus
285 of 1,000-fold. These predictions suggest that for the photodiodes used, which have output
286 optical power when coupled with a plastic fiber of between 25-75 micro Watts, the caustic
287 focus may yield an optical power equivalent to as much as several Watts, at low scattering
288 angles. Measurement of the light intensity generated is beyond the scope of this paper, but
289 instead the utility of the system for making useful molecular measurements was explored
290 experimentally. Overall, it was observed that the limit of detection using SYBR Green I for
291 DNA using a dilution of 1:5,000 was 1 ng/ml, which is comparable to the detection range
292 found in commercial fluorimeters, but with much less instrumentation complexity. Similar
293 performance when measuring alkaline phosphatase using BBT, suggests that the caustic
294 focus using a spheroid sample leads to adequate light intensity for calibration. Also,
295 because the most intense light is limited to a fraction of the sample volume, photobleaching
296 of the available fluorophores is slowed, permitting steady-state fluorescence measurement
297 over longer periods of time. It is important to note that depending the concentration of
298 surface active agents in the sample, the area used to calculate fluorescence using integrated
299 density needs to be guided by the ray tracing shown in Figure 1. In general, as the
300 concentration of surfactant increases, the area used to calculate fluorescence should be
301 smaller and closer to the zone of highest light intensity in the droplet in order to have
302 reproducible fluorescence measurements.

303 Figure 3 shows typical results from the optical system when measuring calf thymus DNA
304 using SYBR Green I, compared to published results by Rengarajan and colleagues [9] The
305 data shown is based on triplicate measurements, and a linear best fit accompanies the data.
306 For the typical SYBR Green I dilution of 1:5,000 from an original solution dissolved in DMSO,
307 the calibration is not linear at DNA concentration much above 1 microgram/ml. It was also
308 useful to see whether the optical platform could yield reasonable detection sensitivities for a
309 variety of cell phone cameras. First-year students at Arizona State University [8] tested the
310 optical platform using their own cell phone cameras to record the images and calibrate the
311 system. In all cases, the images were taken using a side view and processed using ImageJ
312 as described in the experimental section.



313

314 **Fig. 3. Calibration curve for SYBR Green I detection of calf thymus DNA and**
 315 **comparison with published results using a standard fluorimeter [8] (blue dashed line).**
 316 **Each green triangle represents the average of triplicate measurements for a sample.**
 317 **The red dashed line is a linear fit of the data. More calibration data using a variety of**
 318 **smartphones can be found online [9]. For the concentration of SYBR Green I used, it**
 319 **is well known that relative fluorescence is not linear with DNA concentration above 1**
 320 **microgram per ml, and thus higher concentrations are not shown in this figure.**

321

322 3.4 BBT

323

324 Figure 4 shows a sequence of images over time to illustrate the evolution of a fluorescent
 325 species due to enzymatic action by alkaline phosphatase on BBT. As seen in Figure 4a,
 326 before ALP reacts to produce BBT anion, the drop appears relatively dark in the interior with
 327 blue light illuminating from the right side of the image and seen exiting the left side. A
 328 gradual brightening of the drop is evident as the reaction proceeds. A series of
 329 measurements were taken after 7 minutes and compared to the manufacturer's published
 330 results for alkaline phosphatase measurement after 15 minutes. The slopes of the
 331 calibration lines were very similar (1290 RFU/minute versus 1280 RFU/minute) when the
 332 images were processed and referenced to the linear range below 0.8 milliunits.

333

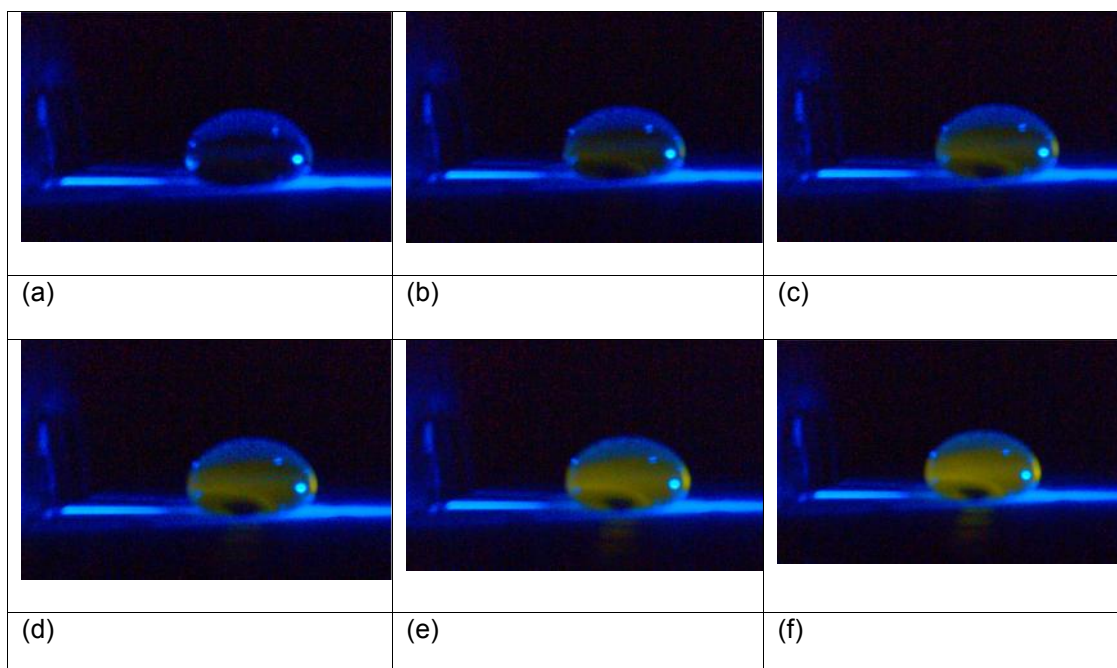
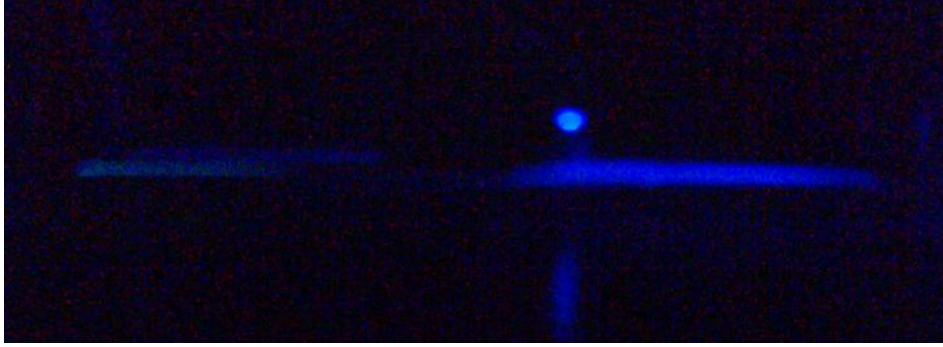


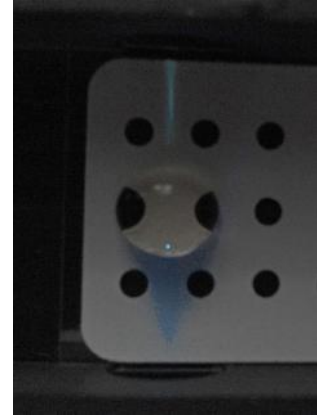
Fig. 4. Sequence of 6 images used in calibrating alkaline phosphatase (ALP) detection using BBT as a substrate. In these images, ALP concentration of 0.16 milliunits is being analyzed. The time sequence of images after combining the substrate with the enzyme are: (a) 0; (b) 1; (c) 2; (d) 3; (e) 5 and (f) 7 minutes. Data were taken at 27 C.

3.5 Tartrazine

Tartrazine fluorescence was discussed briefly in Part I, but for comparison with SYBR Green I and BBT, other observations and measurements are instructive. Figure 5a shows an image of a 0.2 mM tartrazine solution from a side view in a dark room, while Figure 5b shows the same sample with the room lights turned on. Figure 5a does not indicate the presence of fluorescence, but in Figure 5b it appears that the light leaving the sample is in its emission spectra. In fact, using a fiber optic spectrometer (see Figure 6) to detect the light exiting the solution in the forward direction indicates that the light is in the expected emission wavelength band. However, it does appear that the fluorescence intensity is much weaker than SYBR Green I or BBT anion fluorescence since measurement using cell phone imaging is not possible, because the sample appears to have an integrated density similar to the background, even after color filtering.



(a)

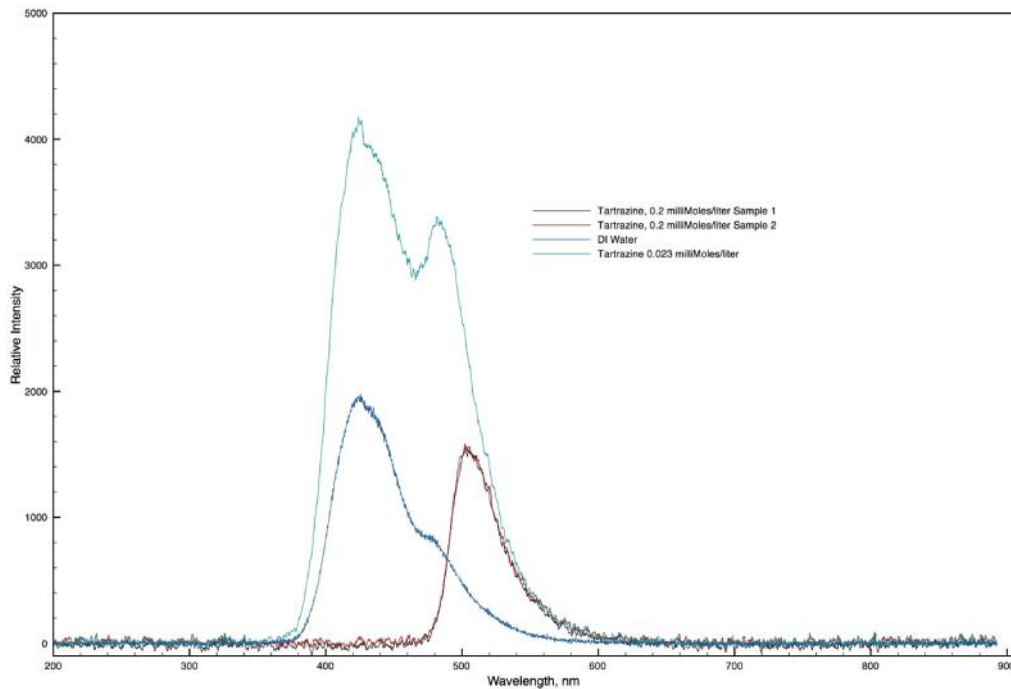


(b)

354

355 **Fig. 5. Images of 0.2 millimoles per liter of tartrazine in water: (a) side view of the**
 356 **sample in the dark with excitation light illuminating the spheroid droplet from the**
 357 **right, (b) top view of the same sample with the lights turned on.**

358



359

360 **Fig. 6. Four spectra recorded by a fiber optic spectrometer for light from a blue LED**
 361 **at 430 nm maximum wavelength exiting an oblate spheroid containing either an**
 362 **aqueous solution of tartrazine or DI water. The green line is the spectra for 0.023**
 363 **millimoles per ml tartrazine, the blue line is for DI water, and the red and black lines**
 364 **are for 2 separate measurements of 0.2 mM tartrazine.**

365 From Figure 6, it is evident that for 0.2 mM tartrazine, the light exiting the sample is
366 significant shifted from the spectra of the Blue LED being recorded leaving a droplet of DI
367 water. For a tartrazine concentration of 0.023 mM, the spectra exiting the sample is seen to
368 have mostly the excitation light, but there is a shoulder detectable that corresponds to the
369 presence of some fluorescence. It should be noted that the intensity recordings vary in
370 Figure 6 since the caustic focus was intentionally not identical in each measurement and
371 hence the spectral shift can be more clearly presented.

372

373 **4. CONCLUSION**

374

375 Newton's zero order caustic can be used to detect fluorescence in a liquid sample, using a
376 low power source, by shaping the sample in order to refract light and create a zone of high
377 intensity light within the sample. In this system, no optical lenses or filters need be
378 employed, which significantly differs from what is commonly found in commercially available
379 instruments. For weakly fluorescent molecules, the beam exiting the sample can be used to
380 detect the emission spectra, especially when the concentration is high enough to also
381 absorb much of the incident excitation light. For strongly fluorescing molecules such as
382 those used to detect dsDNA or for molecules used biological probes, the light exiting the
383 sample shaped into an oblate spheroid can be detected with a digital phone camera and
384 analyzed using software developed for electrophoresis gel quantitation. Changing the major
385 to minor axis in the sample can be used to shift the caustic focus, but reflection and zones of
386 low light intensity should be avoided since they can limit the area for analysis in quantitative
387 measurements.

388

389 **ACKNOWLEDGEMENTS**

390

391 The ASU Foundation provided support for this work and for funding to CH. We are also
392 grateful for the helpful recommendations on the literature of optical caustics in water drops
393 made by Prof. James Andrews at Youngstown State University.

394

395

396

397 **COMPETING INTERESTS**

398

399 Authors declare that no competing interests exist.

400

401 **AUTHORS' CONTRIBUTIONS**

402

403 This work was carried out in collaboration among all authors. Authors AAG and LN
404 designed the study and wrote the first draft of the manuscript. Authors CH and AAG
405 measured and analyzed the results. Authors LN and VM reviewed the optical ray tracing
406 and managed the literature searches. All authors read and approved the final manuscript.

407

408

409

410 **REFERENCES**

411

412 1. Lock JA. Ray Scattering by an arbitrarily oriented spheroid. II. Transmission and cross-
413 polarization effects. Applied Optics. 1996;35(3):515-531.

- 414 2. Lock JA, McCollum TA. Further thoughts on Newton's zero-order rainbow. Am. J. Phys.
415 1994;62:1082-1089.
- 416 3. Chen GQ, Wu YM, Wang J, Zhu T, Gao SM. Fluorescence spectroscopy study of
417 synthetic food colors. Guang Pu Xue Yu Guang Pu Fen Xi. 2009;25:18-2522.
- 418 4. Lock JA, Xu F. Optical caustics observed in light scattered by an oblate spheroid. Applied
419 Optics. 2010;49:1288-1304.
- 420 5. Kofler J. Focusing of light in axially symmetric systems within the wave optics
421 approximation, Master Thesis. University of Linz, Austria; 2004.
- 422 6. Ren KF, Onofri F, Roze C, Girasole T. Vectorial complex ray model and application to two-
423 dimensional scattering of plane wave by a spheroidal particle. Optics Letters. 2011;36:370 -
424 372.
- 425 7. Egri A, Horváth A, Kriszta G, Horváth G. Optics of sunlit water drops on leaves: conditions
426 under which sunburn is possible. New Phytologist. 2010;185:979.
- 427 8. Haynes K. BME103 s2013:Projects2. Open Wet Ware. Accessed 13 June 2013. Available:
428 http://openwetware.org/wiki/BME103_s2013:Projects2.
- 429 9. Rengarajan K, Cristol SM, Mehta M, Nickerson JM. Quantifying DNA Concentrations
430 using fluorimetry: a comparison of fluorophores. Molecular Vision 2002;8:416-421.

431

432 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

433 x_s = distance between a point source and the center of the sample being illuminated

434 b = major axis of a spheroid

435 a = minor axis of a spheroid

436 SYBR Green I = DNA intercalating dye used to measure dsDNA by fluorescence

437 BBT = a substrate for alkaline phosphatase that fluoresces in its anionic form

438 ALP = alkaline phosphatase

439 PTFE = polytetrafluoroethylene

440 PBT = Polybutylene terephthalate

441 Cartesian coordinates = (x, y, z)

442 Radius of spheroid = R

443 light intensity = I

444 OpAmp = operational amplifier

445

446

447

APPENDIX

448