RESEARCH ON LEARNING

Assessing the Effectiveness of a Novel Microscopy Technique in Middle & High School Science Classrooms

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Abstract

Although research and new technologies have introduced different ways of observing microorganisms, including scanning and electron microscopy, these methods are expensive and require equipment that is typically not found in a middle school classroom. The transmission-through-dye technique (TTD; Gregg et al., 2010), a new optical microscopy method that can be used with current basic light microscopes, relies on the fairly simple mechanism of filtered light passing through a dyed medium to produce an image that reflects cell thickness. With this technique, living microorganisms look bright red against a dark background, and movement can be seen easily among dead microorganisms and debris that show up black. Since the technique is low-cost and easy to implement, it addresses the needs of practitioners and is appropriate for a wide array of school contexts. We describe a three-week, hands-on, inquiry-based unit on TTD microscopy for middle and high school students.

Key Words: *transmission-through-dye; TTD microscopy; inquiry.*

○ Introduction

When children begin using microscopes in schools, the door to a new world is opened. What starts as just a drop of water becomes so much more as soon as it's placed on a slide and put under a microscope. Within that drop, thousands of tiny, single-celled microorganisms are alive; however, while microscopy activities can be fascinating, they can also be challenging. The field of vision in a light microscope produces little contrast, and studying microorganisms in nature can be complicated because large amounts of contaminating dead organisms and debris make it difficult to distinguish living things. Middle school lessons on microscopes also tend to be the same every year (McIntosh & Richter, 2007). Although research and new technologies have introduced different ways of observing microorganisms, including scanning and electron microscopy, these methods are expensive and require unique pieces of equipment that are far beyond what is traditionally found in a middle school classroom (Coffey et al., 2015). As such, optical microscopy is the focus in most curricula, but advances with this technique are limited.

Observational microscopy has facilitated biology education since its conception. While methods of microscopic observation have changed and become more advanced, few of these advancements are used in K–12 classrooms. For instance, Harlow and Nilsen (2011) had scientists connect virtually in video conference calls with scientists who had access to scanning electron microscopes; the students were able to see the visuals produced by these microscopes. Hands-on experience with this technology is unrealistic given its cost and lack of accessibility for K–12 students. Adams et al. (2000) also describe a "virtual" model in which scanning electron microscopy is facilitated by postsecondary students and the images accessed online.

Most curricula that have been developed for K–12 microscopy units – for instance, McIntosh and Richter's (2007) flower morphology unit – utilize traditional optical microscopy techniques. New technology, such as portable microscopes that can be paired with smartphones and tablets, are becoming more common, and while this can provide students with ways to see organisms in the field, the image produced is the same as basic light microscopy. Other innovations include integrating gaming elements into light microscopy explorations (Kim et al., 2016) and developing a mobile nanoscience laboratory (Coffey et al., 2015). In our review of the literature, we did not find any techniques to modify traditional optical light microscopy in K–12 classrooms that (a) were inexpensive, (b) could be used by students in a hands-on manner, and (c) provided a different visual than traditional light microscopy. The TTD microscopy technique satisfies all of these characteristics.

In consideration of the above constraints with current microscopy curricula in K–12 settings, we set out to determine whether a microscopy technique developed for postsecondary students and research – namely, transmission-through-dye (TTD) – could be useful for middle school students. TTD microscopy (Gregg et al., 2010)

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uses a simple technique, wherein a sample of cells is placed on a slide and covered in blue dye (Acid Blue 9, a food colorant that is completely nontoxic and can be safely used in the school environment). The dye has a dark blue color and is imaged in red light, which is generated by a small LED-based device. Since these materials are common and do not require the purchase of new technology, the technique is inexpensive. Importantly, Acid Blue 9 is excluded only by intact cells, while dead material absorbs the dye and appears dark on images. The slide is then viewed either through a red filter or by using red illumination. If it is imaged in red light, there is no need for a red filter. As the light from the microscope passes through the dye and the sample, an image is formed that reflects cell thickness. Brighter sections of the image indicate thicker cells, while darker sections of the image indicate thinner cells or parts of cells (see Figure 1; Gregg et al., 2010). TTD adds strong contrast to transparent cells, making them easily visible and distinct from dead matter and debris (see Figure 2; Gregg et al., 2010). This property of the technique can be utilized for studying aquatic microorganisms (Model & Davis, 2016).

In this mixed-methods research study, we set out to determine whether a three-week inquiry-based unit focused on TTD microscopy resulted in differences in middle school students' content test scores, as well as their conceptual understandings of microscopy techniques and microorganisms. In this study, students had opportunities to investigate, learn, and experience two different microscopy techniques as well as evaluate the similarities and differences between those techniques.

○ Study Context

The study took place in a middle school in the northeastern United States (see Table 1 for the school's demographic breakdown). Two classrooms participated in the research project. Since the school was on a schedule with 45-minute blocks, there were 10 block classes involved in the study (four from classroom A and six from classroom B). While classroom A (four block classes) was taught by a single teacher, classroom B (six block classes) was cotaught by two teachers. As such, there were three teachers in this study. A total of 235 students attended class during the intervention. We include qualitative data from lab activities for all student participants; however, for the pretests and posttests, we only include student data that provided both pretest and posttest results for each student. In this sense, the pre-post comparisons that had missing data were excluded from the study. We used listwise deletion to exclude any students who had missing data on the variables of interest in our study. This resulted in 29.6% of the data being excluded from the analysis. Hence, we analyzed a total of 166 valid, matching pretest and posttest scores.

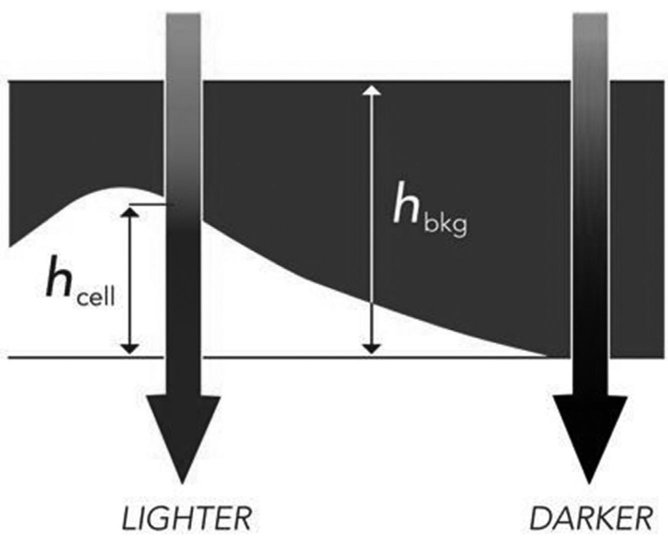

Figure 1. Transmission-through-dye microscopy. Thicker cell sections are more brightly illuminated than thinner cell sections due to the depth of dye.

○ Curriculum

The curriculum unit in this research study was designed to introduce TTD microscopy to students in grade 6. In addition, the unit focused on Protista and plankton. It is made up of three individual lab activities lasting approximately nine days (in 45-minute blocks, for a total of approximately 400 minutes of instructional time). All lab activities are inquiry-based and are aligned to the *Next Generation Science Standards*. In the first activity, students learn how to use a bright-field microscope to view dry-mount slides and identify and describe features of common protozoan species (i.e., algae, diatoms, paramecia). In the second activity, students learn to make wet-mount slides, use the TTD microscopy technique, and identify protists based on movement. In the third activity, students compare and contrast bright-field and TTD microscopy techniques and use both techniques to characterize and identify different Protista species from a mixed culture.

○ Methods

The research questions for this study were as follows: (1) What is the effect of the intervention on students' test scores? (2) Is there a significant ($P < 0.05$) difference in the content exam gains of male and female students? (3) Is there a significant ($P < 0.05$) difference in the content exam gains of students who participate in a single-teacher versus a cotaught classroom? (4) What are students' conceptions of microscopic organisms and microscopy techniques during the lab activities?

Quantitative Data Analysis

In order to evaluate the effectiveness of the TTD technique with students, one member of our team developed and administered a content knowledge test. Example test questions and question types are listed in Table 2. To analyze the data gathered from the pretests and posttests measuring students' content knowledge, we used descriptive statistics, as follows, to test for differences between means and medians. We used the Wilcoxon signed-rank test (Wilcoxon, 1945) to determine whether students' content knowledge changed significantly between pretests and posttests, and the Mann-Whitney *U*-test (Mann & Whitney, 1947) to determine whether there were differences in the content exam gain scores between males and females and between single-teacher and cotaught classrooms. Since the data collected were non-normally

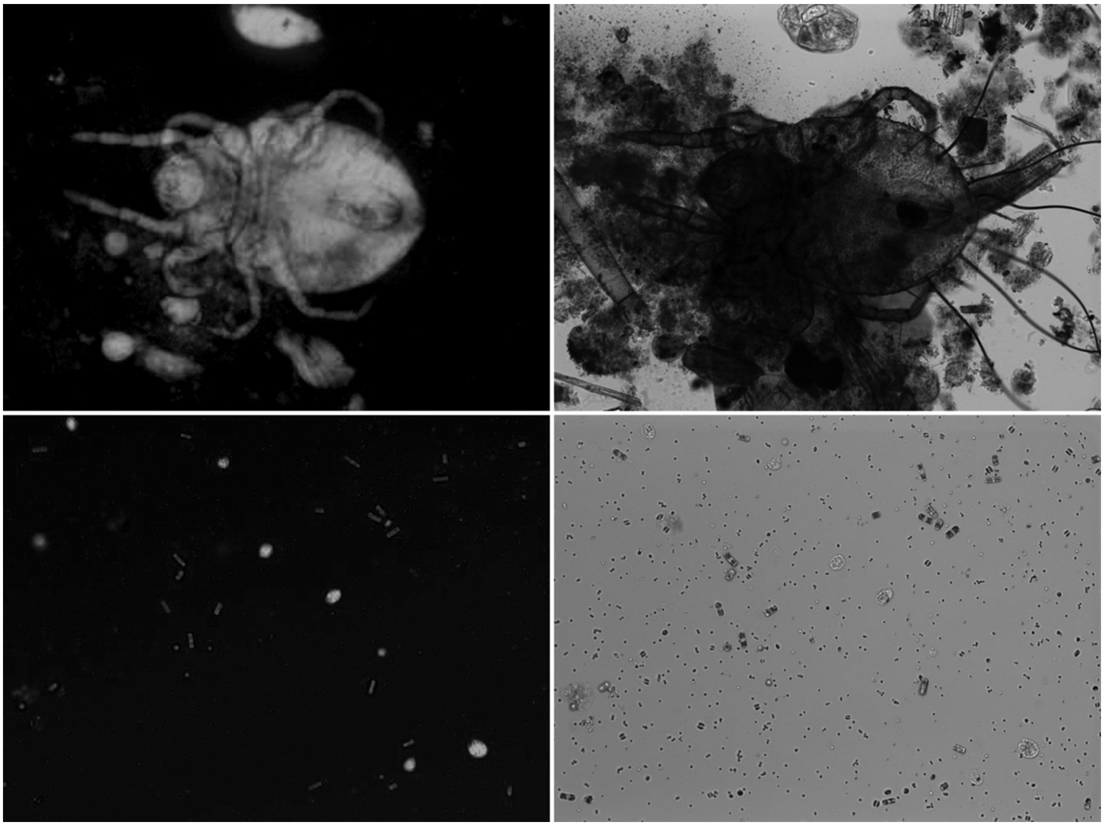

Figure 2. (Left) A colony of algae and a microorganism imaged in red by TTD microscopy. (Right) The same algae and organism imaged in gray by conventional bright-field microscopy. In TTD, the red cells are alive (brightness being proportional to thickness) and the black cells are dead. Live and dead cells cannot be differentiated on the bright-field image.

Table 1. Student demographics of the middle school in this study.

Demographic	*n*	%
Asian Pacific Islander	17	2.1%
Black, Non-Hispanic	132	16.5%
Hispanic	19	2.3%
Multiracial	80	10.0%
White, Non-Hispanic	551	68.9%
Students with Disabilities	161	20.1%
Economically Disadvantaged	800	100%

Source: 2017–2018 Report Card, https://reportcard.education.ohio.gov/school/detail/017251.

distributed, we considered nonparametric tests most appropriate for the analyses. The level of significance was set at $\alpha = 0.01$.

Qualitative Data Analysis

In addition to quantitative data, our team collected and analyzed students' responses to open-ended laboratory questions. Responses were assessed to determine and categorize students' content knowledge. These results were summarized using descriptive statistics. We examined students' responses and focused on revealing patterns and themes that emerged from the data using the content analysis technique (Neuendorf, 2002). Researcher-created descriptive groups and themes were established directly from the open-ended lab questions. Some of the open-ended questions were also analyzed using Wordle, a web-based data visualization tool, which creates word-frequency representations called word clouds (Feinberg, 2014). Once any word emerges more frequently in the written text, a word appears larger in the word-cloud. By triangulating the data collection, the research team managed to determine the overall effectiveness of the TTD technology and its usefulness in middle school science classrooms. In addition, our team explored students' perceptions

Table 2. Example test questions and question types.

Example Test Question	Question Type
What type of slide is used to observe a live specimen using a light microscope?	Multiple-choice
What is the purpose of the cell membrane?	Multiple-choice
If the cell membrane breaks, what would happen to the cell?	Multiple-choice
For the questions that follow, choose the correct name for each organism.	Matching question
Why would a cell appear black when using the transmission-through-dye technique?	Multiple-choice
Using the transmission-through-dye image below, choose the best answer for why some of the cells are bright and some are dim.	Multiple-choice

Table 3. Descriptive statistics and percentiles for students on pretest and posttest.

	N	Mean	SD	25th Percentile	50th Percentile (Median)	75th Percentile
Pretest	166	2.43	1.515	1.00	2.00	3.00
Posttest	166	5.90	2.076	4.00	6.00	8.00

Table 4. Wilcoxon signed-rank test: ranks for students on pretest and posttest.

	N	Mean Rank	Sum of Ranks
Negative Ranks*	9	49.56	446.00
Positive Ranks**	150	81.83	12,274.00
Ties***	7		
Total	166		

Note: *Posttest < Pretest; **Posttest > Pretest; ***Posttest = Pretest.

of microorganisms and their experiences with two microscopy techniques.

○ Results

(1) What is the effect of the intervention on students' test scores? Descriptive statistics for the students' pretest and posttest scores are presented in Table 3. While student means (± SD) for the pretest raw scores were 2.43 ± 1.51, means for the posttest raw scores were 5.90 ± 2.076. The 25th, 50th, and 75th percentiles are also reported in Table 3.

We used a Wilcoxon signed-rank test to determine whether there was a significant difference between students' pre- and post-exam scores. A total of 265 sixth-grade students participated in this three-week study; however, data for only 166 of the participants were suitable for the analyses. Students' content exam scores were measured before the intervention and immediately after the intervention. The following results are reported as medians unless otherwise stated (due to nonparametric analysis). The test results showed that there was a statistically significant difference between students' pretest (2.00) and posttest (6.00) scores ($z = -10.205$, $P = 0.000$, $r = -0.56$). In addition, the sum of the positive difference ranks ($\Sigma R+ = 12{,}274.00$) was larger than the sum of the negative difference ranks ($\Sigma R- = 446.00$), showing that students' content knowledge improved significantly. While 150 of the 166 participants' exam scores increased after the three-week intervention, seven remained the same and nine decreased (Table 4). In addition, the effect size for the matched-pair samples was 0.56. Therefore, our analysis indicates that this three-week intervention helped students improve their content knowledge significantly.

(2) Is there a significant difference in the content exam gains of male and female students? We used a Mann-Whitney *U*-test to answer this question. The distributions of the content exam gain scores for males and females were similar, as assessed by visual inspection. Content exam gain scores were not significantly different between males (3.00) and females (4.00) ($U = 3078.500$, $z = -0.249$, $P = 0.837$).

(3) Is there a significant difference in the content exam gains of students who participate in a single-teacher versus a cotaught classroom? We used a Mann-Whitney *U*-test to answer this question. Distributions of the content exam gain scores of students who participated in a single-teacher versus a cotaught classroom were similar, as assessed by visual inspection. Content exam gain scores were not significantly different between the single-teacher classroom (3.00) and the cotaught classroom (4.00) ($U = 3057.000$, $z = -0.124$, $P = 0.215$).

(4) What are students' conceptions of microscopic organisms and microscopy techniques during the lab activities? We analyzed students' responses to three open-ended questions from the lab activities:

- Do you think anything can live in a drop of water?
- What kinds of creatures or things live in a pond?
- What are the differences between using the TTD technique and the light microscopy technique? Discuss the differences you saw using these two techniques.

The first two questions were asked before the intervention to evaluate students' preconceptions related to microscopic organisms. The last question was asked at the completion of the intervention. Our team analyzed the first two questions using Wordle (Feinberg, 2014). Student responses to the question "Do you think anything can live in a drop of water?" showed that, while most of the students believed some organisms could live in a drop of water, they couldn't elaborate or provide details. Similarly, in response to the second question ("What kinds of creatures or things live in a pond?"), students wrote about macroorganisms, such as fish, frogs, and ducks, indicating very limited knowledge of microscopic organisms. The numeric frequency of the words that students used in their responses are listed in Tables 5 and 6.

Several themes emerged from student responses to the third question regarding differences between using the TTD technique and the basic optical microscopy technique. With regard to the pros of using the TTD technique, one student explained in this representative response:

> In the TTD technique, it was more visible to see, you saw it clearly, you could distinguish the microbes from each other, easier to find and saw movement better. In the light microscopy technique you could barely tell how they moved, which was which, and see where it was

Table 5. Frequency of the words in student responses to the question "Do you think anything can live in a drop of water?"

Word	Frequency in Student Responses
Water	146
Yes	122
Live	108
Drop	85
Think	55
Single	35
Bacteria	34
Small	33
Algae	33
Need	30
Thing	29
Food	27
Oxygen	26
One	25
Pond	24
Organism	24
Something	21
Air	21
Microscope	19

Table 6. Frequency of the words in student responses to the question "What kinds of creatures or things live in a pond?"

Word	Frequency in Student Responses
Fish	152
Frog	117
Tadpole	104
Algae	80
Live	78
Pond	62
Duck	52
Creature	46
Turtle	46
Thing	45
Plant	33
Snake	33
Crawfish	28
Water	25
Kind	23
Spider	20
Creek	20
Toads	19
Pad	19

Table 7. Quantitative analysis of students' responses regarding differences between using the light microscopy technique and using the TTD technique (our question 3).

Based on your observations, what are the differences between using the light microscope technique and using the TTD technique?			
Theme	*N*	%	Example Student Responses
Any advantage of TTD	140	54%	"In the white light technique, the organisms were just very fitting into the background. This made them difficult to see. However, during the TTD technique, the cells were all very bright compared to the background thus making them more visible." "The differences between TTD and the white light is the TTD is red, color shows if an organism is dead or alive, see microbe better, see more movement."
Any advantage of light	27	10%	"White light is true color and realistic." "The white light would only be effective to find the color of the creature."
No answer	51	20%	N/A
Wrong or irrelevant	41	16%	"Because you can't see all those without a microscope, it was cool to see all of those that are in the water from a creek." "It was very awesome and there would be so many organisms that you can see better and a lot of people would say the same."

Note: Responses that included more than one theme were dual-coded; therefore, the number of responses is greater than the number of student participants.

> because they weren't outlined like on the TTD technique. To me, the image was cloudy under the white light.

Some students were more critical of the TTD technique. In this representative comment, a student explains the differences between the techniques:

> The differences between the TTD technique and the light microscopy technique are that light microscopy technique provides the true color, it's more realistic, you can see what they are eating. The TTD technique uses a red light. The color shows if an organism is alive or dead. TTD makes it easier to see microbes better. TTD allows the viewer to see more movement.

In the 235 coded responses, 54% of students reported that the TTD technique provided them some advantages over bright-field microscopy, such as showing clearer pictures, highlighting organism movement, and making it easier to distinguish between living and dead organisms. Approximately 10% of the students favored using basic optical microscopy, since they could see the true color of organisms. A little over a third of students either gave an irrelevant answer or didn't provide a response to the question. Irrelevant answers were defined as an answer that included correct information but was not related to the question being asked. We provide example irrelevant/incorrect answers as well as numeric results in Table 7.

○ Discussion & Study Limitations

In this study, we analyzed both quantitative and qualitative data to determine whether there were meaningful differences in students' content knowledge before and after the intervention, to explore students' perceptions of microorganisms, and to investigate students' experiences with two microscopy techniques. Regarding the impact of this intervention on the students' content knowledge, content-test results revealed that students' content knowledge significantly improved after the intervention, with a moderate effect size of 0.56. There were not any statistically significant differences between male and female students or between cotaught and single-teacher classrooms with regard to student content knowledge. The majority (54%) of students cited the TTD technique as being more useful than basic optical microscopy for observing microorganism movement and making distinctions between living organisms and dead organisms and debris.

There were several limitations in this study. First, it focused on sixth-grade students in a single school. While increasing the number of schools and/or including more grade levels could provide a better opportunity to understand the possible effects of this intervention, the school district asked that we only work with one of the middle schools. Similarly, only two teachers participated in the study. Expanding the number of participating teachers would provide opportunities for researchers to gain a deeper understanding of the implementation challenges of the intervention, as well as to evaluate teachers' perceptions of the technique and its usefulness. Second, there was no control group in this study against which the intervention groups could be compared, which is important for evaluating the impact of a treatment. However, because assessing the effect of the treatment using control-group design would require a period of 16 weeks or longer (Ashburn et al., 2002), it was impossible to implement in the present study. This curricular intervention was relatively short (three weeks of lessons), but it could be expanded upon in the future and adapted for other grade levels. Lastly, note that color-blind students may have extreme difficulty seeing, or may be unable to see, the bright red contrast against a dark background produced by TTD microscopy.

○ Recommendations for Improved Learning

Using microscopy techniques in science classrooms provides many benefits to students and teachers. While technology has improved tremendously and opened up new venues in the past two decades, limited school budgets, lack of relevant curricula, and the extensive training necessary to use these very sensitive, cutting-edge technologies make it nearly impossible to include these instruments in everyday classrooms. In addition, traditional optical light microscopy has its limitations and doesn't satisfy all the requirements that are needed in science classrooms. In this study, we introduced the TTD technique as a solution to these issues. TTD microscopy can provide visuals that are easier to analyze than traditional light microscopy, is inexpensive, and is an easy-to-teach technique that can be used by many students. While the TTD technique can be used independently and successfully in science classrooms, comparing and contrasting the technique with traditional light microscopy can provide opportunities for discussions about the appropriate use of these techniques, leading to deeper understanding. Using TTD and traditional light microscopy, teachers can guide students to consider the benefits and drawbacks of these techniques, moving away from cookbook laboratory studies and supporting a more inquiry-minded approach.

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