

RE: JPAE-17-10, entitled "Innovative Strategies for Clinical Microscopy Instruction: Virtual versus Light Microscopy"

Reviewer Comments:

Reviewer #1: This is a well written and very relevant study that provides innovative ways to teach practical skills. Microscopy is a commonly performed procedure by PAs
Himmerick K, Dehn R, Coplan B, Hooker R. "What Procedures Are Performed by Primary Care Providers?" Presented at: AAPA 2015. May 23-27, 2015.

Additionally, this type of innovation can be modeled for teaching other procedures. The study design, data analysis and interpretation are also relevant and could easily be improved following the comments below:

a) Explain how randomization of students was achieved. Did you assign random numbers to each participant or did you use computer software to randomize?

Taking a randomly generated non-alphabetical list of students and assigning each student to one of four groups achieved randomization. This explanation was included in the first paragraph of the Design section of the Methodology.

b) Show that your randomization was successful in preventing bias and in ensuring that all confounding variables were balanced in the two groups. A demographic table of participants comparing group A vs group B is missing. Such a table could include (mean GPA and standard deviation in courses done prior to the experiment, mean prior clinical experience etc)

The biggest confounding variable would be students who performed higher in previous units of study. Therefore, the authors took final course grades from each of the three previous units of study. Independent sample t-tests for each group (A&B) were calculated for the 3 units prior to the study unit, which showed that randomization was successful in preventing bias and that confounding variables were balanced in the two groups. This explanation and Table 1, Demographics of Study Participants, were included in the Design section of the Methodology.

c) Present your results in means (arithmetic mean) and median (the middle point of a number set, in which half the numbers are above the median and half are below).

For example 60,62,62,70,70,90,90,95

Mean =74.9

Median =70

See corrections to Table 2.

d) Explain in detail your definition and rationale of learning outcomes from a microscopy course for PA students in your program: How you decided on the learning outcomes. Traditionally, the teaching of microscopy in clinical programs has two major aims to: Learn to set up and use a light microscope and ii) to be able interpret the findings. For example, PAs in most primary care offices should be able to set up a microscope and analyze dermatological scrapings, urine samples, sputum, blood etc. This is particularly important for PAs working in rural and underserved areas.

Your study comparing virtual and light microscopy learning outcomes appears to focus on interpreting images and IT DOES NOT address the other important learning outcome ie the ability to be able to prepare a sample and mount it on a microscope, focus and tell what is on the slide. Microscopy is not like X-rays where the technician performs the x-ray, generates the image and the radiologist reads it. Please clarify.

There was, indeed, a learning outcome for assuring the student learned to properly prepare a sample, mount it on a microscope, focus the microscope and then identify what was on the slide. In addition there a second learning outcome that assured the students learned to appropriately interpret the findings of the microscopic study. This information has been added to the paper in the Methodology section under Setting.

e) You state that outcomes were measured by quiz 1 and quiz 2. How sure are you that these quizzes actually measured the intended outcomes? Were these validated instruments? How different was quiz 1 from quiz 2. From a pedagogical and assessment design, at what level of Bloom's taxonomy were these questions?

What about the practical test? Did you follow Millers pyramid or any other assessment framework? These questions are important for future readers of your article to make sure that your assessment tools were actually measuring your intended outcomes.

Quiz questions were linked to specific learning outcomes for the course, thus assuring that quizzes measured intended outcomes. Quiz 1 assessed microscopic cellular components (both blood cells and epithelial cells), while Quiz 2 assessed microscopic images of casts and crystals. Although the quizzes consisted of all new questions prepared by the course director, the item analyses for each exam (generated by the testing software) were studied using the point biserial and p-value for each question. Based on this item analysis, the two quizzes were determined to be equivalent. All quiz questions are categorized as part of the cognitive domain in the knowledge category of Bloom's taxonomy. The practical exam encompasses all four levels of Miller's pyramid: Knows (the microscopic elements), Knows how (to set up a urine microscopic), Shows how (to read a urine microscopic) and Does (sets up, reads, reports and interprets a urine microscopic). These changes were incorporated in the Analysis section of the paper.

d) A practical exercise at the same time of quiz 1 would have been idea.

At the beginning of each lab session in the light microscopy section of the study, students participated in practice sessions to become familiar with the techniques of preparing a sample, mounting it on a microscope, focusing the microscope and identifying what was on the slide. Practice sessions were assessed for all students during the practical exam at the end of the unit. This study focused on comparing learning outcomes between light microscopy and video microscopy, with those students in the light microscopy group receiving instruction in preparing a sample, mounting it on the microscope, focusing the microscope and identifying what was on the slide. This information is included in the Study Protocol section of the paper.

f) In medical education, there is an interest in assessing immediate knowledge achievement and long-term knowledge retention. Can you provide data six months or a year later between the two groups?

Unfortunately, there was no data collected during the clinical year (in particular, in the summative practical exam) on identification of urine microscopic elements for this cohort of students. However, after this study was completed, it was decided to include identification of urine microscopic elements on summative exams for future cohorts.

g) As you stated, study limitations included the single center study design and inability to study several cohorts (prospectively). It would be great to generate more data from forthcoming cohorts.

This idea was discussed, but given the amount of lab time needed (four lab sessions) for this study, the course team decided to reduce the number of lab sessions making prospective analysis difficult.

Other comments:

The students viewed microscopic images on a computer monitor and all students in the class observed the same image at the same time with direction from the instructor. Was it the same instructor for each group?

Yes, the same instructor taught all the students for the entire course. This information was added to the paper in the Setting section.

Restate the conclusion: In a single cohort in one PA program, virtual microscopy enabled students to learn about identification of microscopic cellular components. Taken together data suggests that a combination of both light microscopy and virtual microscopy would be ideal. The impact on long term retention remains to be evaluated.

Conclusion reworded to include this language.

practical exam scores for the previous five cohorts (class of 2011-2015) were pooled and compared to the study cohort (class of 2016). The 67 students in the study group achieved a mean of 94.3% for the practical exam; the 289 students pooled from the previous five cohorts achieved a mean of 90.6% ($t = -4.59$; p). How similar were these cohorts and how did you control for other variable between cohorts?

For analysis of demographics of cohorts for the practical exam, the previous five cohorts and the study cohort were compared using the science GPA upon matriculation, as well as student age, race, and gender. Data was analyzed using independent t-tests for science GPA and age and Fisher's Exact Test for race and gender with no statistical significance identified. This information was added to the Analysis section of the paper.

Provide a note on curriculum for the reader to understand the background of your course design. Is this an integrated curriculum, problem based, organ system course followed by another course. Is it team based learning. When is this course covered relative to other courses? Course sequence is important in determining outcomes

Added to the Setting section of the paper.

Abstract: line 49 of the conclusions: Virtual microscopy is an effective educational strategy, and students prefer this method when learning about interpreting images of clinical specimens. I am not sure if they would prefer virtual modality if the aim was to learn about setting up and utilizing a microscope. This is well captured in a student's comment "I actually prefer a combination of both. The virtual microscopy was better for introduction to the things we needed to know, but as a clinician I need to be competent running the scope myself and identifying them under the scope. This wording highlighted above was added to the abstract, but it may make the abstract have more words than are allowed by the publisher.

Reviewer #2: This paper describes research into student learning differences when virtual vs. light microscopy teaching methods were employed. The paper is well written and readable (thank you). I have two concerns, however -- one relatively minor and one major:

(1) It appears that the entire sample was collected from a single PA program, which limits the generalizability of the authors' conclusions to the population of PA programs.

This information was included in the limitations section of the paper.

(2) The use of multiple t tests inflates alpha. I suggest protecting the t tests with an omnibus ANOVA or apply a Bonferroni or Sidak (or similar) correction to the critical p-value.

Our reading of the paper, ignoring our comparison of the class of 2016 to previous classes, shows we present p-values for (1) Quiz 1, (2) Quiz 2, (3 and 4) paired t-tests, and (5) Group A vs Group B, testing of increase between the 2; so, 5 p-values in total. If we add in the comparison of this group to previous groups (and we would argue that this was not main part of the paper, so those

p-values do not count and are not included in the paper), we would give 6 more p-values. Since the reviewer would like for us to use an adjustment, a smaller alpha, we added the following to the results section of the paper: No correction for multiple testing was applied to outcomes, as those selected for analysis were related and were used to evaluate consistency across findings. P-values <0.05 were considered to be statistically significant.

Other than that, this paper might make for a good brief report, given the sampling issue.

Reviewer #3: Advances in the technology used for student instruction in the health professions moves forward each year. The pre-test, post-test, cross over design has been effectively used to understand the impact of optical versus virtual teaching methods in medical education (see Wilson et al, Med Educ, 2016).

This reference was used in the paper.

The present manuscript is the first report of such an effort applying a near similar study design (no pre-test of knowledge) to understand virtual versus optical microscopy in the teaching of PA students. The manuscript clearly defines the research design and uses appropriate statistical methods to make the needed comparisons. The study adds some information about student preference for instruction using virtual microscopy. In the absence of pre-test of knowledge, the authors chose to determine if selected student characteristics might explain the results but truly does not define differences in knowledge.

The authors are unclear as to what we are being asked to respond to. Please clarify.

Innovative Strategies for Clinical Microscopy Instruction:

Virtual versus Light Microscopy

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Abstract

Purpose: Compare virtual microscopy to light-microscopy to determine differences in learning outcomes and learner attitudes in teaching clinical microscopy to physician assistant (PA) students.

Methods: A prospective, randomized crossover design study was conducted with a convenience sample of 67 first-year PA students randomized to two groups. One group used light-microscopes to find microscopic structures, while the second group used instructor-directed video-streaming of microscopic elements. At mid-point of the study, the groups switched instructional strategies. Learning outcomes were assessed via post-test following each section of the study, with comparison of final practical exam results to previous cohorts. Attitudes about the two educational strategies were assessed by a post-course Likert-scale questionnaire.

Results: Analysis of the first post-test demonstrated students in the video-streamed group had significantly better learning outcomes than the light-microscopy group ($p=0.004$; Cohen's $d = 0.74$). Analysis of the post-test after crossover showed no differences between the two groups ($p=0.48$). Between the two post-tests, students first assigned to the light-microscopy group scored 6.6 mean point increase (± 10.4 SD; $p=0.0011$) while students first assigned to the virtual-microscopy group scored 1.3 mean point increase (± 7.1 SD; $p=0.29$). The light-microscopy group improved more than the virtual-microscopy group ($p=0.019$). Analysis of practical exam data revealed higher scores for the study group compared to five previous cohorts of first year students ($p<0.0001$; Cohen's $d = 0.66$). Students preferred virtual-microscopy to traditional light-microscopy.

Conclusions: Virtual microscopy is an effective educational strategy, and students prefer this method when learning about interpreting images of clinical specimens.

Introduction

Microscopy instruction in allopathic and osteopathic medical schools in the United States has shifted significantly towards the use of virtual microscopy, with 44% of the medical schools surveyed in 2009 using virtual microscopy exclusively compared with only 14% in 2002.¹ These

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4 findings are congruent with the educational advantages of virtual microscopy that have been
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6 identified by Maybury and Farah, including benefits such as “improved collaboration among
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8 learners, and added variety in ways of course delivery”.² With this ever-increasing use of virtual
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10 microscopy, the need to introduce this new technology into medical education curricula is
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12 paramount.³

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16 Previous instruction in microscopy has been carried out through the use of light
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18 microscopes in an instructor-led laboratory session, with each student receiving one-on-one
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20 assistance from the instructor. With increasing class sizes, the use of this hands-on microscopy
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22 instruction results in excessive amounts of instructor time devoted to assisting every student. This
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24 observation is supported by previous studies that identified challenges to hands-on microscopy
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26 instruction, such as curricular reform resulting in fewer laboratory sessions and reduced access to
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28 space and equipment.⁴ These challenges have stimulated several research studies that demonstrate
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30 not only a move to more virtual microscopy,² but also a student preference for virtual learning,^{5,6}
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32 and better student performance on exams after virtual microscopy instruction.⁷

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36 Using virtual microscopy, recent technology advances create the ability to video-stream a
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38 microscopic image from the instructor’s microscope to either a large screen that can be viewed by
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40 all students or to individual monitors (or laptop computers) located at student stations. This
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42 capability allows the instructor to point out specific elements in the microscopic image that might
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44 otherwise be overlooked when only viewed by student observation with a light microscope. This
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46 ability to video-stream and present virtual microscopy from the instructor’s microscope to student
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48 monitors results in more focused instruction for each student while requiring less instructor time,
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50 and allows simultaneous instruction of more than one student.^{4,5}

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54 The pre-clinical year curriculum at the XXX Physician Assistant (PA) Program includes a
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56 course in XXX, which incorporates the microscopic analysis of urine sediment during the renal unit
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58 of study. Instruction centers on classroom-based lectures, which include photomicrographs of urine
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60 microscopic findings, followed by student participation in small group laboratory instruction in
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provider-performed microscopy. In previous years, laboratory instruction consisted of light microscopy performed by the student with assistance from the instructor. The recent implementation of virtual (video-streamed) microscope technology into the curriculum provides the opportunity to compare learning outcomes from traditional light microscopy to learning outcomes from virtual microscopy.

This study explored student learning outcomes and student attitudes after the implementation of virtual microscopy in order to determine 1) if there were identified differences in learning outcomes using virtual microscopy versus traditional hands-on light microscopy, and 2) if attitudes and perceptions indicated a preference for either virtual microscopy or light microscopy instruction.

Methodology

Participants:

The eligible participants for this study, conducted in the 2014-2015 academic year, were the 67 first year students in the XXX course at XXX PA Program. The XXX and the XXX Institutional Review Boards approved the study.

Setting:

The XXX course is a continual part of the Inquiry-Based Learning, organ system-based curriculum at the XXX PA Program, and has been taught by the same instructor for the past 20 years. The two educational strategies (light-microscopy and virtual microscopy) were implemented at the beginning of the renal unit (the last unit of study) in the pre-clinical curriculum of the XXX PA Program. Prior to the renal unit of study, students completed nine months of the pre-clinical curriculum, including units of study covering anatomy and physiology and the following organ systems: Hematology, Dermatology, Endocrinology, Gastroenterology, Cardiology, Pulmonology, Orthopedics, Neurology and Psychology.

Two of the overall learning outcomes of the XXX course is for students to 1) perform basic diagnostic medicine procedures common to the ambulatory care setting and 2) interpret and

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4 evaluate diagnostic test results to determine proper diagnosis and treatment. These learning
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6 outcomes were included in the XXX course for the purpose of teaching students to set up and use a
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8 light microscope, as well as interpret the findings of the microscopic study. This has proven to be
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10 extremely valuable to students over the years, in particular those students working in primary care
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12 clinics in rural and underserved areas.

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15 During the renal unit of study in the XXX course, students are instructed in the
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17 performance of urine sediment microscopy in four distinct laboratory sessions, with different
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19 microscopic elements studied each week and the same instructor for all groups and laboratory
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21 sessions. In addition, students are instructed on the proper set up and use of the light microscopes,
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23 with a learning outcome of preparing a urine sample, mounting it on a microscope slide and placing
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25 it on the microscope stage, focusing the microscope, and identifying the elements in the urine
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27 sediment being studied.

31 **Design:**

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33 The idea of comparing hands-on light microscopy to virtual microscopy has been studied
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35 using two groups of students divided into two phases within a course, so that each group of
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37 students would experience each form of microscopy and learning could be compared.^{8,9,10} Using a
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39 similar prospective, randomized cross-over design for this study provided a more equitable training
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41 experience for all students, as it did not benefit one group over the other, and it reduced inequities
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43 in learning outcomes. This study allowed all students to experience learning through the use of
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45 light-microscopy performed by the student, as well as virtual microscopy under the direction of the
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47 instructor.

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51 Through the use of a randomized controlled crossover comparison study, students
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53 experienced learning through the use of two methods of microscopy instruction. The study was
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55 divided into two parts, Part 1 and Part 2, with the study design outlined in Figure 1.

57 **Figure 1. Crossover Study Design**

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To accommodate course scheduling and space and equipment constraints in the laboratory, the pre-clinical year curriculum coordinator assigned students to one of four laboratory groups (A1, A2, B1 or B2) by taking a randomly generated non-alphabetized student roster and assigning each student to one of the four groups. Approximately 16-18 students were assigned to each group; each group met once per week in a two-hour laboratory session. Group A (lab groups A1 and A2) consisted of 32 students and Group B (lab groups B1 and B2) consisted of 35 students.

In order to assure that randomization was successful in preventing bias and that confounding variables were balanced in the two groups, the authors determined that the biggest confounding variable would be students who performed better in previous units of study. Final course grades from each of the three previous units of study prior to the study unit were analyzed (Table 1). Independent sample t-tests for each group (A&B) were calculated for the three units prior to the study unit, which showed that randomization was successful in preventing bias and that confounding variables were balanced in the two groups. In addition, there were no differences observed in age or gender distribution between the two groups (Table 1).

Table 1. Demographics of Study Participants

In the light microscopy method, the students used the light microscope to independently locate and evaluate microscopic elements with the instructor available for one-on-one assistance. In the virtual microscopy method, the students viewed microscopic images on a computer monitor and all students in the class observed the same image at the same time with direction from the instructor.

During Part 1 of the study, Group A received light microscopy instruction and Group B received virtual microscopy instruction. At the mid-point of the study, the instructional strategies were switched for each group. In Part 2 of the study, Group A received virtual microscopy instruction and Group B received light microscopy instruction.

We assessed learning outcomes using a post-test after each section of the study (Part I: Quiz 1 and Part 2: Quiz 2) and by a practical exam at the end of the course. We assessed student

attitudes about the two educational strategies using a post-course Likert-scale questionnaire designed for this study.

Study Protocol:

Prior to each laboratory session, all students experienced the same didactic sessions covering urine microscopic components.

Study Part 1: During the first two laboratory sessions, learning outcomes focused on identification of microscopic cellular components (blood cells and epithelial cells) found in freshly prepared urine sediment slides. All students in Group A received laboratory instruction using light-microscopy and identified microscopic structures with the aid of textbooks and instructor assistance during laboratory sessions, followed by a self-directed microscopic activity that included preparing a sample, mounting it on the microscope, focusing the microscope and identifying what was on the slide. Students in Group B received laboratory instruction using virtual microscopy, with microscopic elements video-streamed to student monitors and direction from the instructor in identifying the microscopic structures, followed by the same self-directed microscopic activity as the students in Group A. At the end of the two Part I laboratory sessions, all students completed a 20-question computer-based multiple-choice examination (Quiz 1). Due to the limitations of presenting images in the testing software used, the questions were printed on a paper handout with microscopic images displayed in the root of the question. Students recorded answers to questions in the computer-based examination software program. Quiz 1 assessed learning outcomes for identification of all the microscopic cellular components covered during Part I of the study.

Study Part 2 (Crossover): During the second two laboratory sessions, learning outcomes focused on identification of microscopic casts and crystals found in freshly prepared urine sediment slides. Group A received laboratory instruction using virtual microscopy and Group B received laboratory instruction using light microscopy. At the end of the Part 2 laboratory sessions, all students completed a 20-question computer-based multiple-choice examination (Quiz 2). As in Quiz 1, the questions were printed on a paper handout with microscopic images displayed in the

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4 root of the question, and students recorded answers to questions in the computer-based examination
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6 software program. Quiz 2 assessed learning outcomes for identification of all the microscopic casts
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8 and crystals covered during Part 2 of the study.
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11 At the end of the course, all students participated in a hands-on practical exam, which was
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13 administered in the laboratory using light microscopy for identification of microscopic elements.
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15 Students in previous academic years participated in this practical exam when hands-on light-
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17 microscopy instruction was the only instructional method. These data allowed the comparison of
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19 results from the students in the study with the results from students in previous years. We
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21 compared descriptive statistics from the practical exam given during the current study with
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23 descriptive statistics from practical exams given during the five previous student cohorts in order to
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25 assess differences in learning outcomes.
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29 At the end of the study, all students received an anonymous questionnaire, developed by
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31 the course director, in order to determine student attitudes about the light-microscopy and the
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33 virtual microscopy instructional methods. The course director advised the students that they were
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35 not required to complete the questionnaire and that there would be no repercussions for not
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37 completing the questionnaire.
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40 **Analysis:**

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42 Student post-tests (Quiz 1 and Quiz 2) followed Part I and Part II of the study and a hands-
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44 on practical exam was administered at the end of the course. The two post-tests (Quiz 1 and Quiz
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46 2) were required components of the XXX course, contributed to the overall XXX course grade, and
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48 student answers were submitted via a testing software program. Student grades for the post-tests
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50 were calculated by the testing software and then de-identified by the course director. The hands-on
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52 practical exam was a required component of the XXX course, contributed to the overall XXX
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54 course grade, and was submitted using a student identification number in order to blind student
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56 identification to the course director for grading purposes. We analyzed the de-identified student test
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58 data (Quiz 1, Quiz 2, practical exam) using SAS, version 9.4 ©2013 SAS Institute, Inc., Cary, NC.
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4 Quiz 1 and Quiz 2 consisted of new questions, which were categorized in the cognitive
5 domain of Bloom's taxonomy in the knowledge category. Questions were in the multiple choice
6 question format and included a stem consisting of a question to identify a particular part of a
7 microscopic image of urine sediment and five answer choices. The quiz was analyzed using the
8 point biserial and p-value for each question in order to establish the validity of the questions used.
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10 The practical exam encompassed all four levels of Miller's pyramid: Knows (the microscopic
11 elements), Knows how (to set up a urine microscopic), Shows how (to read a urine microscopic)
12 and Does (sets up, reads, reports and interprets a urine microscopic).¹¹
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22 We performed independent t-tests to compare the two groups for possible differences in
23 scores for the Part I, Part II, and practical exams, as well as the differences in change in scores
24 between the Part I and II exams. Additionally, using each student as their own control, we assessed
25 the change between tests within each group using paired t-tests. We compared the practical exam
26 scores from the study cohort to five previous years using independent t-tests, first comparing each
27 individual prior year to the study cohort and then pooling the five years of scores and comparing
28 the combined score with the study cohort's. Cohen's *d* was calculated as an estimate of effect size.
29 For analysis of demographics of cohorts for the practical exam, the previous five cohorts and the
30 study cohort were compared using the science GPA upon matriculation, as well as student age,
31 ethnicity, and gender. Data was analyzed using independent t-tests for science GPA and age and
32 Fisher's Exact Test for ethnicity and gender with no statistical significance identified.
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46 We analyzed the student questionnaire responses to identify attitudes about the two forms
47 of microscopy instruction. The questionnaire consisted of seven questions about learning
48 preferences, with responses based on a five-point scale of Strongly Agree, Agree, Neutral,
49 Disagree, or Strongly Disagree. The questionnaire also included space for open-ended comments.
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51 The Pre-clinical Year Curriculum Coordinator sent an email message to the students that included a
52 link to the SurveyMonkey® questionnaire. Respondent IP addresses were not collected to ensure
53 anonymity; this is the standard process for end-of-course student satisfaction questionnaires.
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Students responded to the questionnaire on a voluntary basis, and we asked students to complete the questionnaire within two weeks. Students received two reminder emails during the two-week period.

Results

Of the 67 students in this study, 32 students were randomized to Group A (composed of lab groups A1 and A2) and 35 students were randomized to Group B (composed of lab groups B1 and B2).

Results for Quiz 1, Quiz 2 and the Practical Exam are provided in Table 2.

Table 2. Group Statistics for Quiz 1, Quiz 2, and Practical Exam

Part 1: Students in Group A (light microscopy) achieved a mean score on Quiz 1 of 88.6%. Students in Group B (virtual microscopy) achieved a mean score on Quiz 1 of 94.9%. Group B outperformed Group A ($p=0.004$; Cohen's $d = 0.74$).

Part 2 (crossover): Students in Group A (virtual microscopy) achieved a mean score on Quiz 2 of 95.2%. Students in Group B (light microscopy) achieved a mean score on Quiz 2 of 96.1%. Both groups performed well and the comparison of the test results was not statistically significant ($p=0.47$).

Comparison of the change between Quiz 1 and Quiz 2 within each group, with each student serving as his or her own control, revealed that Group A students had a mean increase of 6.6 points (± 10.4 SD, $p=0.0011$), while Group B students had a mean increase of 1.3 points (± 7.1 SD, $p=0.29$). Group A improved at a significantly higher rate than Group B between Quiz 1 and Quiz 2 ($p=0.019$).

Analysis of practical exam data demonstrated significantly higher scores for the study cohort (class of 2016) as compared with the previous five cohorts (class of 2016 versus class of 2015, $p<0.0001$; class of 2016 versus class of 2014, $p=0.0013$; class of 2016 versus class of 2014, $p=0.0029$; class of 2016 versus class of 2012, $p=0.0011$; class of 2016 versus class of 2011, $p<0.0001$). Consequently, practical exam scores for the previous five cohorts (class of 2011-2015) were pooled and compared to the study cohort (class of 2016). The 67 students in the study group

achieved a mean of 94.3% for the practical exam; the 289 students pooled from the previous five cohorts achieved a mean of 90.6% ($t = -4.59$; $p < 0.0001$; Cohen's $d = 0.66$).

No correction for multiple testing was applied to outcomes, as those selected for analysis were related and were used to evaluate consistency across findings. P-values < 0.05 were considered to be statistically significant.

Table 3 presents the results of the student questionnaire, which surveyed student attitudes about instructional strategies. Results were compiled based on student responses of agree, disagree, or neutral. We received a 90% response rate (60 of 67 students). Of the respondents, 98% indicated that virtual microscopy was an effective method of learning. One student commented: "When using the video-streamed [virtual] microscopy, we were able to understand what exactly differentiates different types of cells. As a group we were able to understand what we were responsible for learning exactly as it was pointed out to us rather than as individuals searching within a slide set without a clear understanding what other students are seeing/learning. Also, as a group process, it was more time efficient." Only 43% of students agreed that both light microscopy and virtual microscopy were equally acceptable methods of learning, but as one student noted, "I actually prefer a combination of both. The virtual microscopy was better for introduction to the things we needed to know, but as a clinician I need to be competent running the scope myself and identifying them under the scope. So I think a combination method would actually be the ideal [method of learning]." Students overwhelmingly preferred (92%) the virtual microscopy and noted in their comments that "video streaming made it easier to be sure that the professor and I were talking about the same thing at the same time."

Table 3. Student Attitude Survey Responses

Discussion

Data analysis of Part 1 of the study revealed that students who participated in virtual microscopy (Group B) performed significantly better on Quiz 1 than students who participated in light microscopy (Group A). Following the crossover, student in both groups performed similarly

on Quiz 2, i.e., no statistically significant difference in mean scores, and both Group A and Group B performed better on Quiz 2 than they did on Quiz 1. It is of particular interest that students who participated first in the light microscopy followed by the virtual microscopy (Group A) improved their scores at a significantly higher rate than students who participated first in the virtual microscopy followed by the light microscopy (Group B). This may be the result of the improved instruction using virtual microscopy or may reflect that students who had not performed as well on the first quiz committed themselves to more intense study prior to the second quiz.

At the end of the XXX course, students are given a practical exam in which they are required to perform several diagnostic studies of urine samples, including microscopic analysis. This same exam has been administered for the past six years. Comparison of the last five cohorts to the class of 2016 cohort in this study revealed that the class of 2016 cohort scored significantly higher on the practical exam than any of the previous cohorts. This is a strong indicator that incorporation of the virtual microscopy methods into the laboratory portion of the XXX course resulted in improved learning outcomes.

The assessment of student attitudes at the end of this study revealed that students overwhelmingly preferred virtual microscopy in order to better understand the microscopic elements they were responsible for learning, perhaps indicating that students prefer more teacher-centered learning. Virtual microscopy provides a better learning environment for the students than the traditional light microcopy method, which requires individual students to search within a microscopic sample, often without a clear understanding of what they were seeing and learning. Students also recognized that the virtual microscopy provides a more time efficient process for learning microscopic elements in a lab setting with other students who are in competition for the instructor's time. While students were not in favor of abandoning the process of learning light microscopy techniques for use in clinical practice, they felt that a combination of both light microscopy and virtual microscopy would be ideal.

Limitations:

Several possible limitations are apparent in this study. First, the study was done with a single cohort of students at XXX PA Program. This cohort may not be a representative sample of PA students at other institutions, or even in other cohorts at the XXX PA Program, as the study group size is quite small when compared with the number of first-year PA students in the US and the total number of students in all cohorts of the XXX PA Program.

Another limitation may be that there was no pre-test to determine if the student cohorts analyzed for the practical exam data were similar in content knowledge at baseline. However, we compared the study cohort (class of 2016) to the previous five cohorts (class of 2011-2015), and found no statistical significance in the analysis of science GPA, age, gender, and ethnicity. This provided evidence that all six cohorts were comparable.

Conclusion

In previous studies, Mione et al ⁹ and Carlson et al ¹⁰ compared outcomes between light microscopy and projected images for teaching histology and hematopathology. While their findings did not demonstrate a significant difference between the two teaching methods, Carlson et al revealed a student preference for the projection method over the light microscopy method. ¹⁰ However, in a recent meta-analysis, Wilson et al concluded that students who were taught using virtual microscopy performed slightly better than students who were taught using optical microscopy, and students preferred this learning method. ¹² For this current study involving a single cohort at the XXX PA Program, virtual microscopy enabled students to learn about identification of microscopic cellular components. Taken together data suggests that a combination of both light microscopy and virtual microscopy would be ideal. The impact on long-term retention remains to be evaluated.

One consideration when implementing an instructional shift to virtual microscopy would be the potential reduction of formal instruction in independently locating microscopic images using light microscopy, which may challenge the use of light microscopes by PAs in clinical practice. Another consideration would be the feasibility of the implementation of virtual microscopy at other

PA or health profession programs in the US. Access to technology for providing video-streamed virtual microscopy capabilities is expensive and acquisition of the technology may be hampered by budgetary restraints.

As a result of this study, instructional strategies for the XXX course in the XXX PA Program have been modified to reflect a more blended learning methodology. This blended learning methodology includes instruction using video-streamed virtual microscopy to introduce students to the elements found in microscopic analysis of urine sediment, while incorporating instruction in the use of light microscopy to provide students with the microscopy skills needed for clinical practice.

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Figure

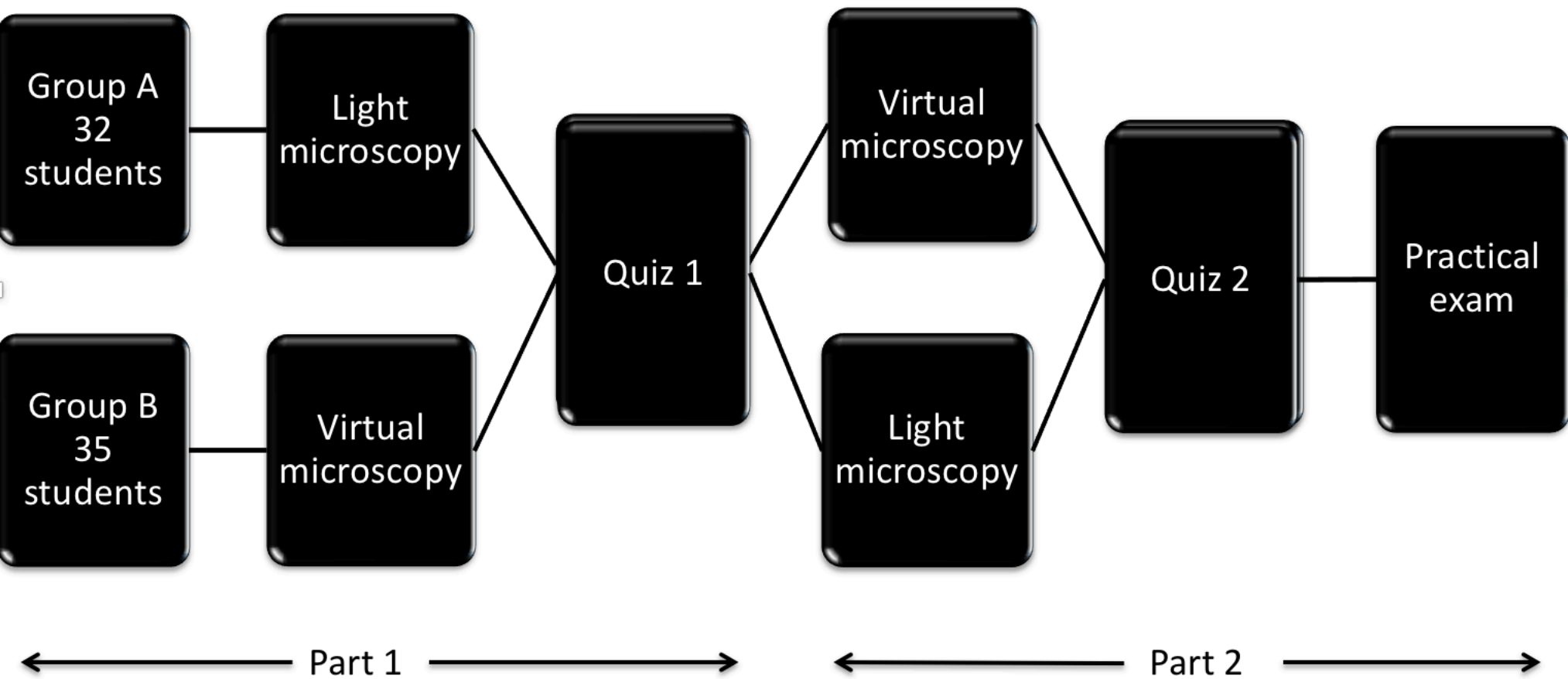


Table 1. Demographics of Study Participants

	Group	N	Mean	SD	<i>t</i> test value	<i>P</i> value
Unit A Final Grade	1	32	92.8	3.1	-1.10	0.27
	2	35	93.7	3.5		
Unit B Final Grade	1	32	90.1	5.0	-1.91	0.062
	2	35	92.1	3.4		
Unit C Final Grade	1	32	90.7	4.7	0.35	0.73
	2	35	90.3	4.2		
Age	1	32	25.3	4.8	0.40	0.69
	2	35	24.8	4.4		
Gender	1	32	75% F	---	---	0.36
	2	35	86% F	---		

Table 3. Student Attitude Survey Responses

	Question	Agree	Neutral	Disagree
1	Using light microscopy for identifying urine microscopic elements was an effective method of learning for me	37 (62%)	13 (22%)	10 (16%)
2	Using virtual (video-streamed) microscopy for identifying urine microscopic elements was an effective method of learning for me	59 (98%)	0	1 (2%)
3	Time allowed for the light-microscopy training was adequate	50 (83%)	8 (13%)	2 (4%)
4	Time allowed for the virtual (video-streamed) microscopy training was adequate	58 (96%)	2 (4%)	0
5	Independently viewing elements in the microscopic image with the instructor available for assistance made the light-microscopy method preferable	14 (23%)	14 (23%)	32 (54%)
6	Having the instructor point out specific elements in the microscopic image made the virtual (video-streamed) microscopy method preferable	55 (92%)	5 (8%)	0
7	Both the light microscopy and the virtual (video-streamed) microscopy were equally acceptable methods of learning for me	26 (43%)	11 (18%)	23 (39%)

Table 2. Group Statistics for Quiz 1, Quiz 2, and Practical Exam

	Group A (n = 32)		Group B (n = 35)		<i>t</i> test value	<i>P</i> value
	Mean % (+/- SD)	Median	Mean % (+/- SD)	Median		
Quiz 1	88.6 (+/- 9.9)	90.0	94.9 (+/- 6.9)	95.0	-3.03	0.004
Quiz 2	95.2 (+/- 5.5)	95.0	96.1 (+/- 5.6)	100.0	-0.73	0.467
Practical Exam	94.9 (+/- 5.1)	96.0	93.7 (+/- 5.0)	95.0	0.97	0.337