Reviewer #1: Major Revision

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?

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)

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

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 undeserved 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.

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 frame work? These questions are important for future readers of your article to make sure that your assessment tools were actually measuring your intended outcomes.

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

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?

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.

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?

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.

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?

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

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.

Reviewer #2: Major Revision

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.
(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.

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

Reviewer #3: Accept

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). 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.