

Innovative Strategies for Clinical Microscopy Instruction: Virtual Versus Light Microscopy

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Purpose The purpose of the study was to compare virtual microscopy with 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 2 groups. One group used light microscopes to find microscopic structures, whereas the other group used instructor-directed video streaming of microscopic elements. At the midpoint of the study, the groups switched instructional strategies. Learning outcomes were assessed via posttest after each section of the study, with comparison of final practical examination results to previous cohorts. Attitudes about the 2 educational strategies were assessed through a postcourse questionnaire with a Likert scale.

Results Analysis of the first posttest demonstrated that students in the video-streamed group had significantly

better learning outcomes than those in the light microscopy group ($P = .004$; Cohen's $d = 0.74$). Analysis of the posttest after crossover showed no differences between the 2 groups ($P = .48$). Between the 2 posttests, students first assigned to the light microscopy group scored a 6.6 mean point increase (± 10.4 SD; $p = .0011$), whereas students first assigned to the virtual microscopy group scored a 1.3 mean point increase (± 7.1 SD; $p = .29$). The light microscopy group improved more than the virtual microscopy group ($P = .019$). Analysis of practical examination data revealed higher scores for the study group compared with 5 previous cohorts of first-year students ($P < .0001$; Cohen's $d = 0.66$). Students preferred virtual microscopy to traditional light microscopy.

Conclusion Virtual microscopy is an effective educational strategy, and students prefer this method when learning to interpret images of clinical specimens.

INTRODUCTION

Microscopy instruction in allopathic and osteopathic medical schools in the United States has shifted significantly toward 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 findings are congruent with the educational advantages of virtual microscopy that have been identified by Maybury and Farah,² including benefits such as "improved collaboration among learners, and added variety in ways of course delivery." With this ever-increasing use of virtual microscopy, the need to introduce this new technology into medical education curricula is paramount.³

Previous instruction in microscopy has been conducted with light microscopes in an instructor-led laboratory session, with each student receiving one-on-one assistance from the instructor. With increasing class sizes, hands-on microscopy instruction results in excessive amounts of instructor time devoted to assisting every student. This observation is supported by previous studies identifying challenges to hands-on microscopy instruction, such as curricular reform resulting in fewer laboratory sessions and reduced access to space and equipment.⁴ These challenges have stimulated several

research studies that demonstrate not only a move to more virtual microscopy² but also a student preference for virtual learning^{5,6} and better student performance on examinations after virtual microscopy instruction.⁷

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

The preclinical year curriculum at the Wake Forest School of Medicine (WFSM) physician assistant (PA) program includes a course in Clinical and Diagnostic Skills (CDS), which incorporates the microscopic analysis of urine sediment during the renal unit of study. Instruction centers on classroom-based lectures, which include photomicrographs of urine microscopic findings, followed by student participation in small group laboratory instruction in 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

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traditional light microscopy to learning outcomes from virtual microscopy.

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

METHODS

Participants

The eligible participants for this study, conducted in the 2014 to 2015 academic year, were the 67 first-year students in the Clinical and Diagnostic Skills (CDS) course at the WFSM PA program. The WFSM and the University of New England institutional review boards approved the study.

Setting

The CDS course is a continual part of the inquiry-based learning, organ system-based curriculum at the WFSM PA program; the same instructor has taught the course for 20 years. The 2 educational strategies (light microscopy and virtual microscopy) were implemented at the beginning of the renal unit, which was the last unit of study in the preclinical curriculum of the WFSM PA program. Before the renal unit of study, students completed 9 months of the preclinical curriculum, including units of study covering anatomy, physiology, and the following organ systems: hematology, dermatology, endocrinology, gastroenterology, cardiology, pulmonology, orthopedics, neurology, and psychology.

Two of the overall learning outcomes of the CDS course are for students to (1) perform basic diagnostic medical procedures common to the ambulatory care setting, and (2) interpret and evaluate diagnostic test results to determine proper

diagnosis and treatment. These learning outcomes were included in the CDS course for the purpose of teaching students to set up and use a light microscope, as well as to interpret the findings of the microscopic study. This instruction has proven to be valuable to students over the years, particularly those students working in primary care clinics in rural and underserved areas.

During the renal unit of study in the CDS course, students are instructed in the performance of urine sediment microscopy in 4 distinct laboratory sessions, with different microscopic elements studied each week and the same instructor for all groups and laboratory sessions. In addition, students are instructed on the proper setup and use of light microscopes, with a learning outcome of preparing a urine sample, mounting it on a microscope slide and placing it on the microscope stage, focusing the microscope, and identifying the elements in the urine sediment being studied.

Design

The idea of comparing hands-on light microscopy to virtual microscopy has been studied using 2 groups of students divided into 2 phases within a course, so that each group of students would experience each form of microscopy, and then the learning outcomes could be compared.⁸⁻¹⁰ Using a similar prospective, randomized crossover design for this study provided a more equitable training experience for all students. It did not benefit one group over the other, and it reduced inequities in learning outcomes. This study allowed all students to experience learning through the use of light microscopy performed by the student, as well as virtual microscopy (via video streaming) under the direction of the instructor.

Through the use of a randomized controlled crossover comparison study, students experienced learning through the use of 2 methods of microscopy instruction. The study was divided into part 1 and part 2. Figure 1 outlines the study design.

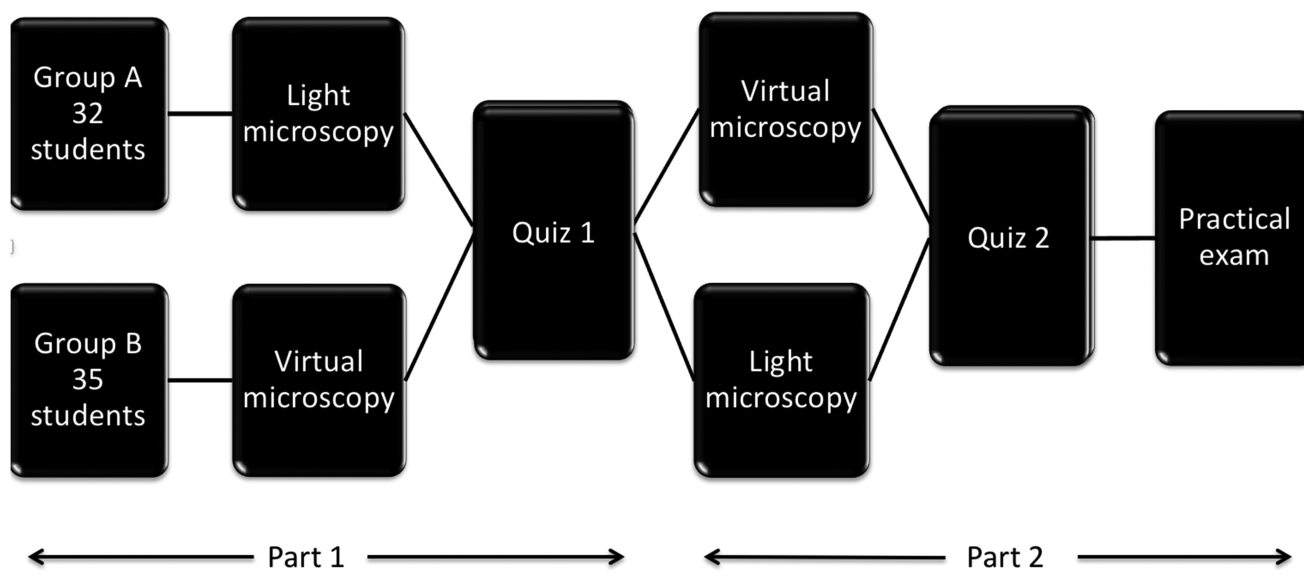


Figure 1. Crossover study design

To accommodate course scheduling and space and equipment constraints in the laboratory, the preclinical year curriculum coordinator assigned students to one of 4 laboratory groups (A1, A2, B1, or B2) by taking a randomly generated, nonalphabetized student roster and assigning each student to one of the 4 groups. Approximately 16 to 18 students were assigned to each group; each group met once per week in a 2-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.

To ensure that randomization was successful in preventing bias and that confounding variables were balanced in the 2 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 3 previous units of study before the renal unit of study were analyzed for group A and group B (Table 1). Independent sample *t*-tests were calculated and showed that randomization was successful in preventing bias and that confounding variables were balanced in the 2 groups. In addition, there were no differences observed in age or sex distribution between the 2 groups (Table 1).

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 individual monitors. All students 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 midpoint 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.

The authors assessed learning outcomes using a posttest after each section of the study (part 1: quiz 1 and part 2: quiz 2) and a practical examination at the end of the course. We assessed student attitudes about the 2 educational strategies using a postcourse, Likert-scale questionnaire designed for this study.

Study Protocol

Before each laboratory session, all students experienced the same didactic sessions covering urine microscopic compo-

nents. During the first 2 laboratory sessions (part 1), learning outcomes focused on identifying microscopic cellular components (blood cells and epithelial cells) found in freshly prepared urine sediment slides. The 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 stage, focusing the microscope, and identifying what was on the slide. The students in group B received laboratory instruction using virtual microscopy, with microscopic elements video-streamed to individual 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 2 laboratory sessions in part 1, all students completed a 20-question, computer-based, multiple-choice examination (quiz 1). Because of the limitations of presenting images in the testing software used, the questions were printed out, with microscopic images displayed in the stem of the question. The students recorded their answers in the computer-based examination software program. Quiz 1 assessed learning outcomes for identifying all the microscopic cellular components covered during part 1 of the study.

During the second 2 laboratory sessions (part 2, crossover), learning outcomes focused on identifying microscopic casts and crystals found in freshly prepared urine sediment slides. The students in group A received laboratory instruction using virtual microscopy, and the students in group B received laboratory instruction using light microscopy. At the end of the 2 laboratory sessions in part 2, all students completed a 20-question, computer-based, multiple-choice examination (quiz 2). The questions were printed out, with microscopic images displayed in the stem of the question. The students recorded their answers in the computer-based examination software program. Quiz 2 assessed learning outcomes for identifying all the microscopic casts and crystals covered during part 2 of the study.

At the end of the course, all students participated in a hands-on practical examination, which was administered in the laboratory using light microscopy for identifying microscopic elements. Students in previous academic years participated in this practical examination when hands-on light microscopy instruction was the only instructional method. These data allowed the comparison of results from the students in the study with the results from

Table 1. Demographics of Study Participants

	Group	N	Mean	SD	t-test Value	P
Unit A, final grade	1	32	92.8	3.1	-1.10	.27
	2	35	93.7	3.5		
Unit B, final grade	1	32	90.1	5.0	-1.91	.062
	2	35	92.1	3.4		
Unit C, final grade	1	32	90.7	4.7	0.35	.73
	2	35	90.3	4.2		
Age	1	32	25.3	4.8	0.40	.69
	2	35	24.8	4.4		
Sex	1	32	75% F	—	—	.36
	2	35	86% F	—		

students in previous years. The authors compared descriptive statistics from the practical examination given during the current study with descriptive statistics from practical examinations given during the 5 previous student cohorts to assess differences in learning outcomes.

At the end of the study, all students received an anonymous questionnaire, which was developed by the course director, to determine student attitudes about the light microscopy and the virtual microscopy instructional methods. The course director advised the students that they were not required to complete the questionnaire and that there would be no repercussions for not completing the questionnaire.

Analysis

Student posttests (quiz 1 and quiz 2) followed part 1 and part 2 of the study, and a hands-on practical examination was administered at the end of the course. The posttests (quiz 1 and quiz 2) were required components of the CDS course and contributed to the overall course grade; student answers were submitted via a testing software program. Student grades for the posttests were calculated by the testing software and then deidentified by the course director. The hands-on practical examination was a required component of the CDS course and contributed to the overall course grade, and was submitted using a student identification number to blind student identification to the course director for grading purposes. The authors analyzed the deidentified student test data (quiz 1, quiz 2, and practical examination) using SAS.¹¹

Quiz 1 and quiz 2 consisted of new questions that were categorized in the cognitive domain of Bloom's taxonomy in the knowledge category. Questions were multiple choice and included a stem consisting of a question to identify a particular part of a microscopic image of urine sediment and 5 answer choices. The quiz was analyzed using the point biserial and *P* value for each question to establish the validity of the questions used. The practical examination encompassed all 4 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).¹²

The authors performed independent *t*-tests to compare the 2 groups for possible differences in scores for part 1, part 2, and practical examinations, as well as the differences in change in scores between part 1 and 2 examinations. In addition, using each student as his or her own control, we assessed the change between tests within each group using paired *t*-tests. We compared the practical examination scores from the study cohort to the previous 5 cohorts using independent *t*-tests, first comparing each individual prior year to the study cohort and then pooling the 5 years of scores and

comparing the combined score with the study cohort. Cohen's *d* was calculated as an estimate of effect size. For analysis of demographics of cohorts for the practical examination, the previous 5 cohorts and the study cohort were compared using the science grade point average (GPA) on matriculation, as well as student age, ethnicity, and sex. Data were analyzed using independent *t*-tests for science GPA and age, and Fisher's exact test for ethnicity and sex, with no statistical significance being identified.

The authors analyzed the student questionnaire responses to identify attitudes about the 2 forms of microscopy instruction. The questionnaire consisted of 7 questions about learning preferences, with responses based on a 5-point scale of agree, neutral, or disagree. The questionnaire also included space for open-ended comments. The preclinical year curriculum coordinator sent an email message to the students that included a link to the SurveyMonkey questionnaire. Respondent internet protocol addresses were not collected to ensure anonymity, which is the standard process for end-of-course student satisfaction questionnaires. The students responded to the questionnaire on a voluntary basis and were asked to complete the questionnaire within 2 weeks. Students received 2 reminder emails during the 2-week period.

RESULTS

Of the 67 students in this study, 32 students were randomized to group A, comprising laboratory groups A1 and A2, and 35 students were randomized to group B, comprising laboratory groups B1 and B2. Table 2 shows the results of quiz 1, quiz 2, and practical examination.

For part 1, quiz 1, the students in group A (light microscopy) achieved a mean score of 88.6%. The students in group B (virtual microscopy) achieved a mean score of 94.9%. Group B students outperformed group A students (*P* = .004; Cohen's *d* = 0.74).

For part 2 (crossover), quiz 2, the students in group A (virtual microscopy) achieved a mean score of 95.2%. The students in group B (light microscopy) achieved a mean score of 96.1%. Both groups of students performed well, and the comparison of the test results was not statistically significant (*P* = .467).

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 (± 0.4 SD, *p* = .0011), whereas group B students had a mean increase of 1.3 points (± 7.1 SD, *p* = .29). Group A students improved at a significantly higher rate than group B students between quiz 1 and quiz 2 (*P* = .019).

Analysis of practical examination data demonstrated significantly higher scores for the study cohort (class of 2016) compared with the previous 5 cohorts (class of 2016 vs class of 2015, *P* < .0001; 2016 vs 2014, *P* = .0013; 2016 vs 2013, *P* = .0029;

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

	Group A (n = 32)		Group B (n = 35)		t-test Value	P
	Mean, % (\pm SD)	Median	Mean, % (\pm SD)	Median		
Quiz 1	88.6 (\pm 9.9)	90.0	94.9 (\pm 6.9)	95.0	-3.03	0.004
Quiz 2	95.2 (\pm 5.5)	95.0	96.1 (\pm 5.6)	100.0	-0.73	0.467
Practical examination	94.9 (\pm 5.1)	96.0	93.7 (\pm 5.0)	95.0	0.97	0.337

2016 vs 2012, $P = .0011$; 2016 vs 2011, $P < .0001$). Consequently, practical examination scores for the previous 5 cohorts (2011–2015) were pooled and compared with the study cohort (2016). The 67 students in the study group achieved a mean of 94.3% for the practical examination; the 289 students pooled from the previous 5 cohorts achieved a mean of 90.6% ($t = -4.59$; $P < .0001$; 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. $P < .05$ 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. The response rate was 90% (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 of what other students are seeing/learning. Also, as a group process, it was more time efficient.

Only 43% of respondents 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.”

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 those who participated in light microscopy (group A). Following the crossover, students in both groups performed similarly on quiz 2—ie, no statistically significant difference in mean scores—and students in 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 in light microscopy first (group A) improved their scores at a significantly higher rate than those who participated in virtual microscopy first (group B). This rate 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 before the second quiz.

At the end of the CDS course, students are given a practical examination in which they are required to perform several diagnostic studies of urine samples, including microscopic analysis. This same examination has been administered for the past 6 years. Comparison of the last 5 cohorts with the class of 2016 cohort in this study revealed that the class of 2016 cohort scored significantly higher on the practical examination than any of the previous cohorts. This result is a strong indicator that incorporation of the virtual microscopy methods into the laboratory portion of the CDS course resulted in improved learning outcomes.

The assessment of student attitudes at the end of this study revealed that students overwhelmingly preferred virtual microscopy 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 students than the traditional light microscopy method, which requires individual students to search within a microscopic sample, often without a clear understanding of what they are seeing and learning. The students in the class of 2016 also recognized that virtual microscopy provides a more time-efficient process for learning microscopic elements in a laboratory setting with other students who are in competition for the instructor's time. Although the students were not in favor of abandoning the process of learning light microscopy

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%)

techniques for use in clinical practice, they believed that a combination of both light microscopy and virtual microscopy would be ideal.

Limitations

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

Another limitation may be that there was no pretest to determine whether the student cohorts analyzed for the practical examination data were similar in content knowledge at baseline. However, the authors compared the study cohort (class of 2016) to the previous 5 cohorts (classes of 2011–2015) and found no statistical significance in the analysis of science GPA, age, sex, and ethnicity. This finding provided evidence that all 6 cohorts were comparable.

CONCLUSION

In previous studies, Mione et al⁹ and Carlson et al¹⁰ compared outcomes between light microscopy and projected images for teaching histology and hematopathology. Although their findings did not demonstrate a significant difference between the 2 teaching methods, Carlson et al¹⁰ revealed a student preference for the projection method over the light microscopy method. However, in a recent meta-analysis, Wilson et al¹³ 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. For the current study involving a single cohort at the WFSM PA program, virtual microscopy enabled students to learn about identifying microscopic cellular components. Taken together, data suggest that a combination of both light microscopy and virtual microscopy would be ideal. The effect 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 United States. Access to technology for providing video-streamed virtual microscopy capabilities is expensive, and acquiring the technology may be hampered by budgetary restraints.

As a result of this study, instructional strategies for the CDS course in the WFSM 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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