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引入虛擬顯微鏡結合卷積神經網絡的方法，應用於組織學實驗課的教學和學習

(配合課程名稱/Course Name)

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一. 報告內文(Content)(至少 3 頁)

1. 研究動機與目的(Research Motive and Purpose)

Is it possible to allow students and teachers to observe microscopic images to allow effective mutual discussion? Is there a way to reduce the teacher's burden when more after school assignments are given to students for students' self-learning? Is it possible to use the CNN computer programs for automatic detection of microscopic structures for histology laboratory teaching and learning?

2. 文獻探討(Literature Review)

Histology is the study of cells and tissue structure of the normal human body and is also known as microscopic anatomy. **Histology lecture** course and **Histology laboratory (practical)** course are two complementary courses. The microscopic structures of the body organs are described during the histology lecture course followed by identifying tissue components in the histology laboratory course. The learning practice of students is traditionally based on identifying microscopic structure of glass slides with optical microscopy in the histology laboratory course.

Recent advancement of digital slide scanner, computer programs and internet techniques lead to virtual tissue slides 虛擬組織玻片observed practically by virtual microscopy (**VM**虛擬顯微鏡). Recently, VM system has been adopted as clinical training and education tools in hospitals and schools (Ginley et al., 2019; Hermsen et al., 2019; Husmann et al., 2009; Krippendorf and Lough, 2005; Mione et al., 2013; Triola and Holloway, 2011; Vainer et al., 2017; Wilson et al., 2016). Clinical application of the VM system includes cytology (Solberg, 2012), hematology (Brueggeman et al., 2012) and pathology (Eraña-Rojas et al., 2017; Ginley et al., 2019; Helle et al., 2013; Hermsen et al., 2019; Kumar et al., 2004; Vainer et al., 2017). The VM system has also applied in histology (Husmann et al., 2009; Krippendorf and Lough, 2005; Mione et al., 2013; Scoville and Buskirk, 2007; Tian et al., 2014; Triola and Holloway, 2011; Vainer et al., 2017; Wilson et al., 2016).

Artificial intelligence (**AI**) computer programs based on algorithms integrated with multi-layer artificial neural networks have been used as deep learning techniques to solve complex problems such as decision-making in thousand-year-old game of Go and facial recognition (Gibney, 2016; Silver et al., 2016). The algorithms and computer programs of convolutional neural network (CNN), the forms of AI, have been applied in object segmentation and object recognition (Ronneberger et al., 2015). CNN computer programs have also applied in histology (Djuric et al., 2017; Komura and Ishikawa, 2018). CNN computer programs have been used in cancer classification, including breast cancer, thoracic tumors, lung cancer, prostate cancer and skin cancers (Litjens et al., 2016; Suleymanova et al., 2018; Wang et al., 2016; Wang et al., 2017; Yang et al., 2020). Recently, CNN computer programs have been shown to be useful for pathological diagnosis or histopathological tissue scoring of fibrosis and inflammation (Heinemann et al., 2018) and kidney histopathologic assessment (Hermsen et al., 2019).

3. 研究問題(Research Question)

The adoption of the interactive virtual microscopy (**VM**) system and convolutional neural network (**CNN**) approach has recently been used in educational and clinical studies. The proposal aims to introduction of VM combined with CNN approach for histology laboratory teaching and learning.

4. 研究設計與方法(Research Methodology)

The course organization of Histology lecture and laboratory at KMU

Both histology lecture and laboratory courses were offered 2 credits for one semester. The histology lecture course provided 16 lectures of 2 hours. Tissues and organ systems were systematically, weekly described in the histology lecture course. The histology laboratory course also provided 16 sections of 4 hours. Within a couple days after the histology lecture, a histology laboratory section was conducted using virtual microscopy (VM) for a practical of the microscopic contents demonstrated in the histology lecture. Weekly contents of learning goals and keywords including microscopic structures and cells, were also presented by teaching assistants before each laboratory section. Then students observed virtual slides with VM. Three teaching assistants and one teacher were in laboratory sections to explain and answer students' questions.

Student participants

In the year 2018 to 2020, virtual microscopes and virtual tissue slides were available to sophomore medical students of the HL class.

Virtual tissue slides and virtual Microscopy

Digital virtual tissue slides數位虛擬組織玻片 were stored in a database server and can be acquired on internet with VM software. The images were viewed via Aperio ImageScope software on computers in general laboratory or computer classrooms at KMU. The experimental team consisted of four students and students used virtual microscopes in turn and demonstrated microscopic structure of tissues to team members.

Supplemental assignments as active learning subjects

To access the student's learning, students were asked to do the assignments of identification of microscopic structures during observation virtual tissue slides. Students accessed virtual slides, identified microscopic structures, marked with a circle while using a computer mouse and submitted the annotated images to the office (to the assigned email address).

Automatic detection of the microscopic structure in digital images via convolutional neural network training

The annotated, representative images for microstructures (basophil, eosinophil, pseudostratified ciliated columnar epithelium, skeletal muscle, and smooth muscle) from students' submission were shown Fig. 1. The microscopic images were determined as right (R) or wrong (W) images of microstructures by teachers. There were 698 images classified into five groups based on microstructures: basophil (48R, 92 W), eosinophil (104 R, 36 W), pseudostratified ciliated columnar epithelium (139 R, 3 W), skeletal muscle (L.S.) (137 R, 1 W), and smooth muscle (L.S.) (137 R, 1 W). The present studies tested the effects of image number in the training set on the image recognition performance of CNN models. There were two different image numbers (138-142 vs 698 images) in the training sets.

For the 1st serial experiments, the right (R) and wrong (W) images of one specific microstructure were divided randomly into two sets: training (70%; 96-99 images) and validation (30%; 42 images), total 138-142 images. For the 2nd serial experiments, the R images of one specific microstructure and all images from five file folders as W images, together 698 images, were divided randomly into two groups: training (70%; 488 images) and validation (30%; 210 images). Both training and validation sets did not have a fixed ratio between right and wrong images for each CNN training program.

The R and W images of one specific microstructure, as a training data set of the specific microstructure, were used to train CNNs to extract each microstructure features and these features were automatically approved in the validation data sets. The CNN parameters were optimized by the validation data sets. The training program was run for 200 epochs (the repeated processes over the training set) to check accuracy of the specific microstructure model. After training, the CNN of microscopic structures was converted to a fully automatic CNN for specific microscopic structures. CNNs of microscopic structure detection were developed in Python 3.7 using the deep learning library Keras based on Google's Tensorflow library. The convolutional neural network layers were shown in Table 1 (Appendix).

Accuracy assessment of CNN models

After training, the CNN models for specific microscopic structures were tested with three additional test-sets of R and W images according to the chosen microstructure. Additional test-sets were not used during the training of CNN models and these images were randomly separated into three groups, 25 images/group. To assess the accuracy performance of the CNN models to detect microscopic structures, I adopted the F1 measure, accuracy (the result of the CNN prediction being identical with the correct classification) and the area under the curve of receiver operating characteristic (AUC) [the area under the ROC curve (AUC)].

Student perception about histology laboratory classes using VM and CNN

An anonymous and voluntary survey about student perceptions of histology laboratory classes using VM and supplemental assignments of microscopic structures was conducted via a questionnaire. The questionnaires were composed of 10 questions (Table 2, Appendix). Answers were provided on five-point Likert scales: (A) Strongly agree (B) Agree (C) Neither agree or disagree (D) Disagree (E) Strongly disagree. After the second examination until the end of the semester, students were invited to answer the questionnaires.

5. 教學暨研究成果(Teaching and Research Outcomes)

(1) 教學過程與成果

The present studies tested the effects of image number in the training set on the image recognition performance of CNN models. Two serial experiments were conducted on different image numbers in the training set: 138-142 images in the first (1st) and 698 images in the second (2nd) serial experiments. The R and W images, as a training data set of the specific microstructure, were used to train CNNs to extract specific microstructure features and these features were automatically approved in the validation data sets. The parameters of CNN models were optimized by the validation data sets during the training programs of 200 epochs. The learning curve of microstructure CNN models displayed the accuracy of the training and validation data vs. the epochs (Fig 2). Then, the CNN programs of these microscopic structures were converted to fully automatic CNNs to identify specific microscopic structures. After training, the CNN models for specific microscopic structures were also tested with three additional test-sets of R and W images.

In the present studies, five CNN models for microstructures (basophil, eosinophil, pseudostratified ciliated columnar epithelium, skeletal muscle, and smooth muscle) were constructed based on virtual slides such as shown Fig. 1. The performance of the CNN models to detect microscopic structures was quantified by F1 measure, accuracy and the area under the curve of receiver operating characteristic (AUC). And the representative figures of accuracy performance of CNN models were shown in Figs. 3 & 4. The present studies also compared the image recognition performance of CNN models carried out by two different image number of images in the training set. The results showed that the image recognition performance of CNN models (eosinophil, skeletal muscle, and smooth muscle) of the 2nd serial experiments with 698 images was significantly higher than the 1st serial experiments with 138-140 images (Fig. 5). The F1 scores of identification of eosinophil, skeletal muscle, and smooth muscle via CNN models were 0.75-0.84. However, there was not significant difference for basophil and pseudostratified ciliated columnar epithelium.

Eighty two 2nd year medical students answered the survey for a response rate of 58% (82/141). The results of the student ratings are shown in Table 1. The reliability statistics score, Cronbach's alpha, of the questionnaire used for the feedback survey was 0.84. The results of the survey showed that students easily adapt to virtual microscope technology. The submission of assignments (pictures of identifying microstructures) are helpful for learning histology. VM is both facilitated to learn histology for students and convenient for discussion within student groups or with the teacher. The data also indicate that the course of applying VM has improved their communication and self-learning skills.

(2) 教師教學反思

Virtual sections with one section plane through the body organs only show the fixed viewing angles of tissue sections and may cause loss of recognition of normal variation among glass tissue slides (Wilson et al., 2016). To display the normal variation of microscopic structures of tissue and organs of the human body, it is necessary to have various virtual sections displaying different section images of the same tissue and organs. In addition, more virtual slides are required to prevent fixed viewing angles of virtual slides and to allow various viewing angles of microstructures. Accordingly, virtual slides and VM provide off-campus learning histology via internet everywhere and every time. Indeed, the digitization of tissue sections of glass slides is an essential for telepathology in disease diagnosis (Madabhushi and Lee, 2016).

The results showed that the image recognition performance of CNN models (eosinophil, skeletal muscle, and smooth muscle) of the 2nd serial experiments with 698 images was significantly higher than the 1st serial experiments with 138-140 images. However, there was not significant difference for (basophil and pseudostratified ciliated columnar epithelium). For microstructures (eosinophil, skeletal muscle, and smooth muscle), the more images in the training data set indeed improve the prediction accuracy. For microstructures (basophil and pseudostratified ciliated columnar epithelium), therefore the more images are required to be included in the training set. Alternatively, CNNs are to be replaced by different programs such as Mask R-CNN (He, 2018).

(3) 學生學習回饋

The majority of students agreed or strongly agreed with the statements, indicating VM is facilitated to learn histology and convenient for discussion within student groups or with the teacher. The majority of students agreed or strongly agreed with the statements, indicating the course of applying VM has improved their communication and self-learning skills. The majority of students also agreed or strongly agreed with the statement, indicating VM and the submission of

assignments (pictures of identifying microstructures) are helpful for learning histology.

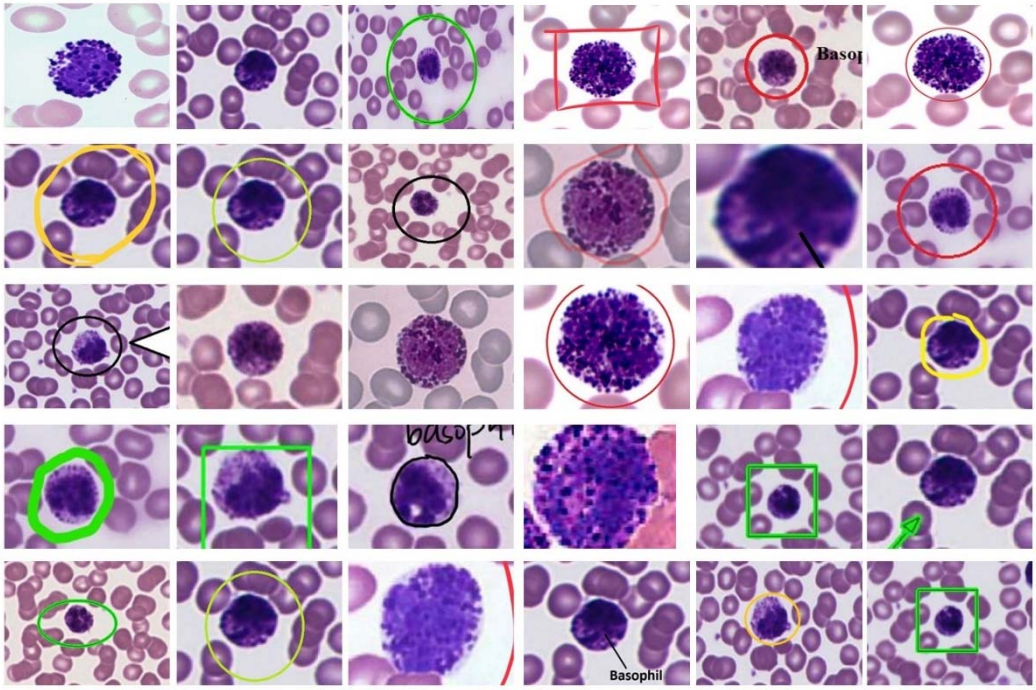


Fig. 1a Representative basophil images, in the training data sets of the basophil CNN models, were used to extract microscopic structure features of basophils to construct the basophil CNN models.

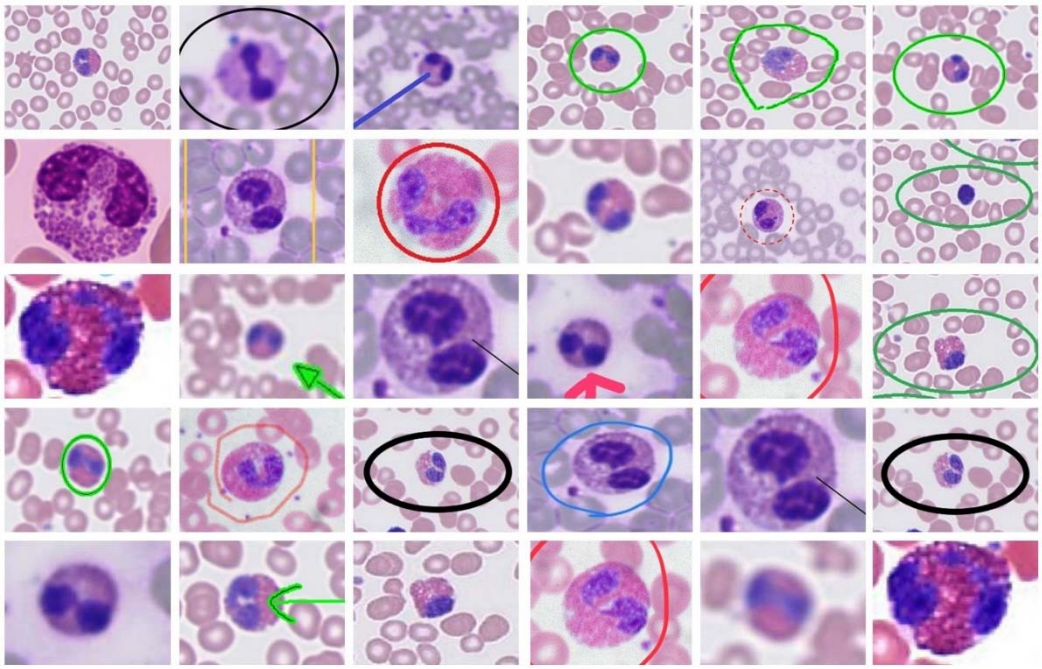


Fig. 1b Representative eosinophil images, in the training data sets of the eosinophil CNN models, were used to extract microscopic structure features of eosinophils to construct the eosinophil CNN models.

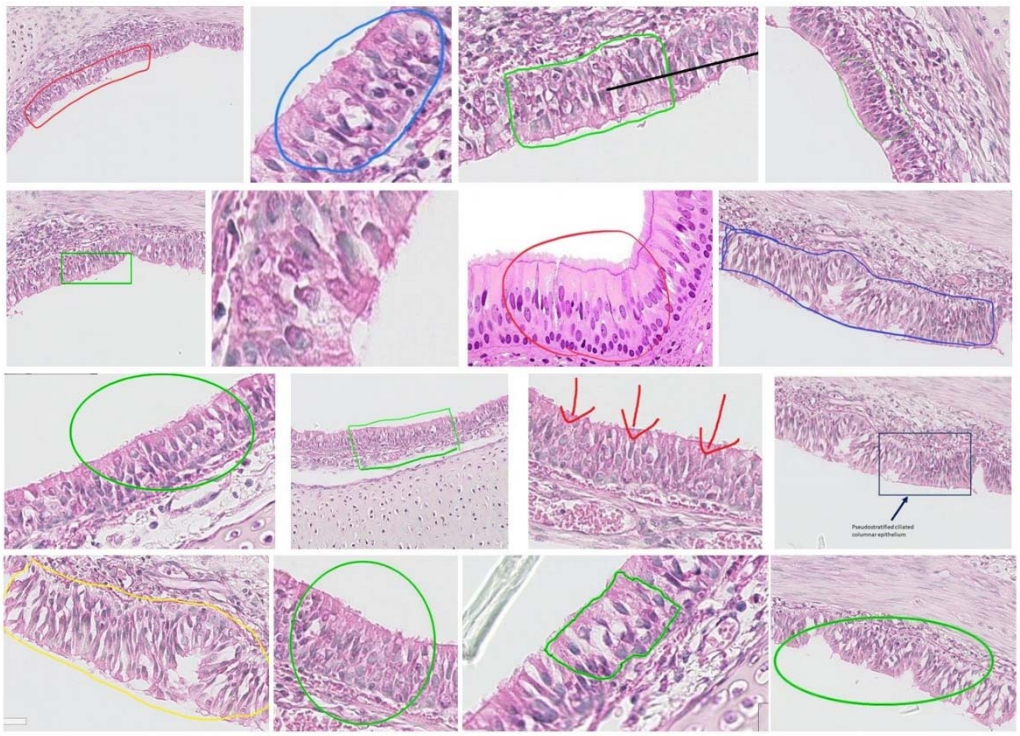


Fig. 1c Representative pseudostratified ciliated columnar epithelium (PSCCE) images, in the training data sets of the PSCCE CNN models, were used to extract microscopic structure features of PSCCE to construct the PSCCE CNN models.

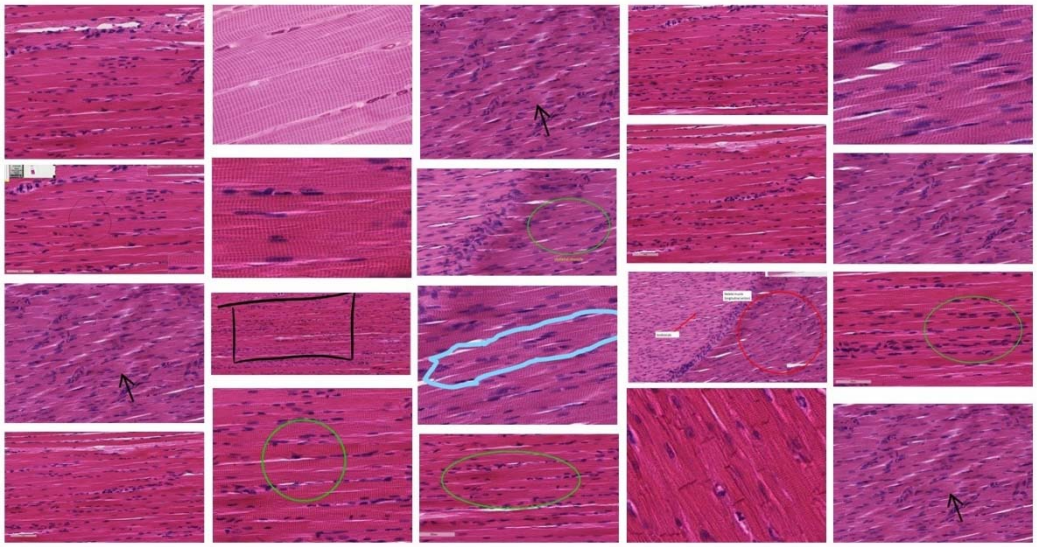


Fig. 1d Representative skeletal muscle images, in the training data sets of the skeletal muscle CNN models, were used to extract microscopic structure features of skeletal muscle to construct the skeletal muscle CNN models.

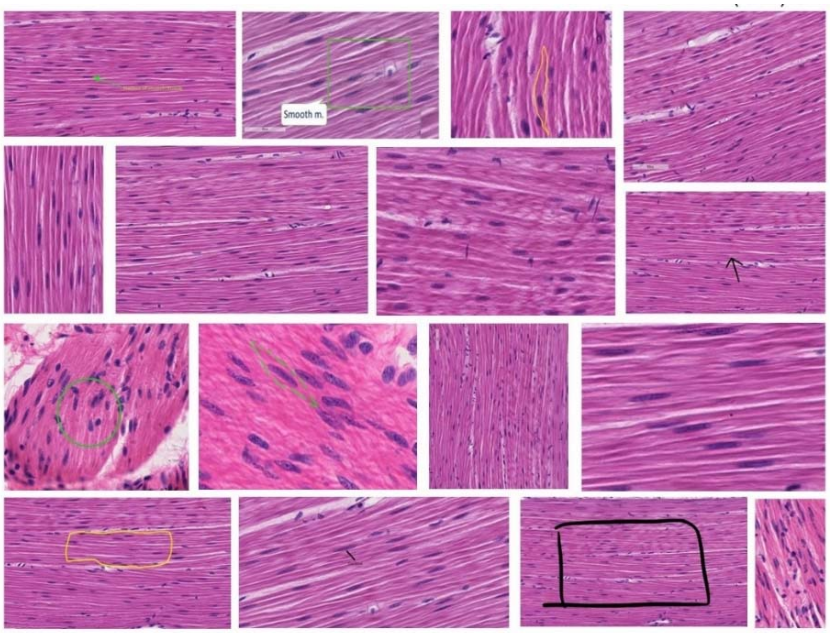


Fig. 1e Representative smooth muscle images, in the training data sets of the smooth muscle CNN models, were used to extract microscopic structure features of smooth muscle to construct the smooth muscle CNN models.

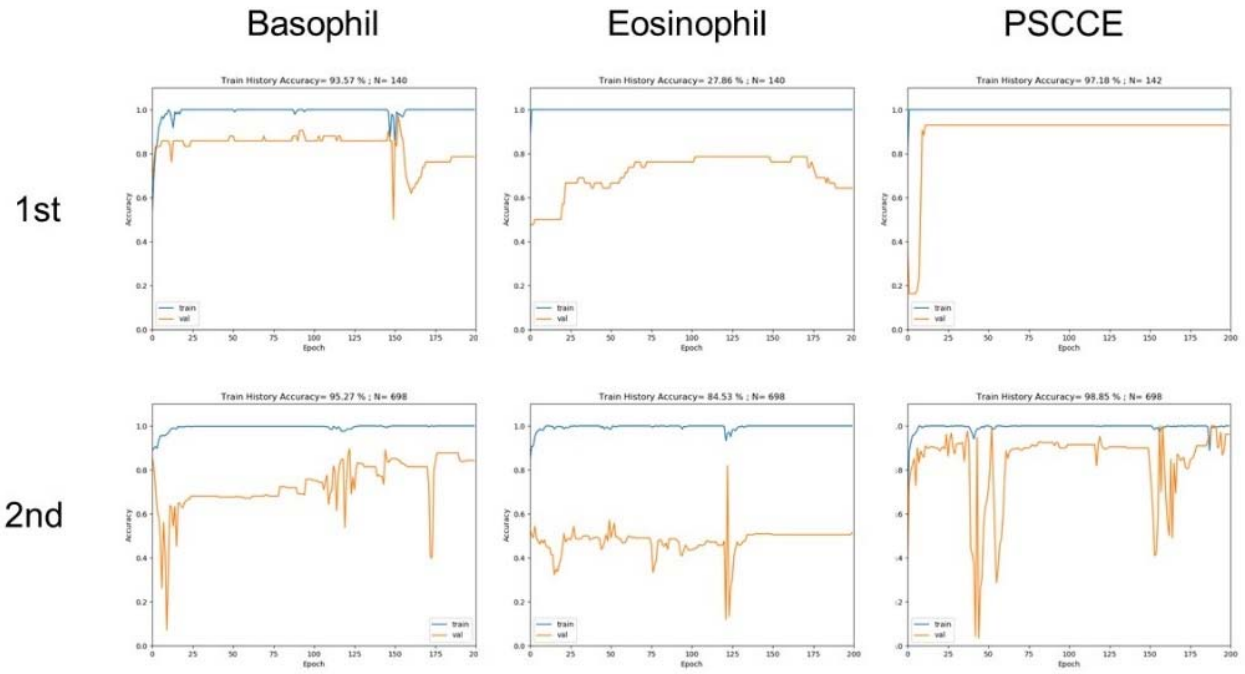


Fig 2a. Representative learning curves of CNN Models of basophil, eosinophil, and pseudostratified ciliated columnar epithelium (PSCCE) from two serial experiments (the first, 1st and the second, 2nd). The right and wrong images (shown in Fig. 1) of one microstructure (ex. basophil), as a training data set of basophil, were used to train CNNs to extract each microstructure (ex. basophil) features and these features were automatically approved in the validation data sets. The training program was run for 200 epochs to check accuracy of the microstructure model (ex. basophil). During training, the CNN parameters were optimized by the validation data sets. Y axis: CNN accuracy of the training (train) and validation (val) data; X axis: the epochs.

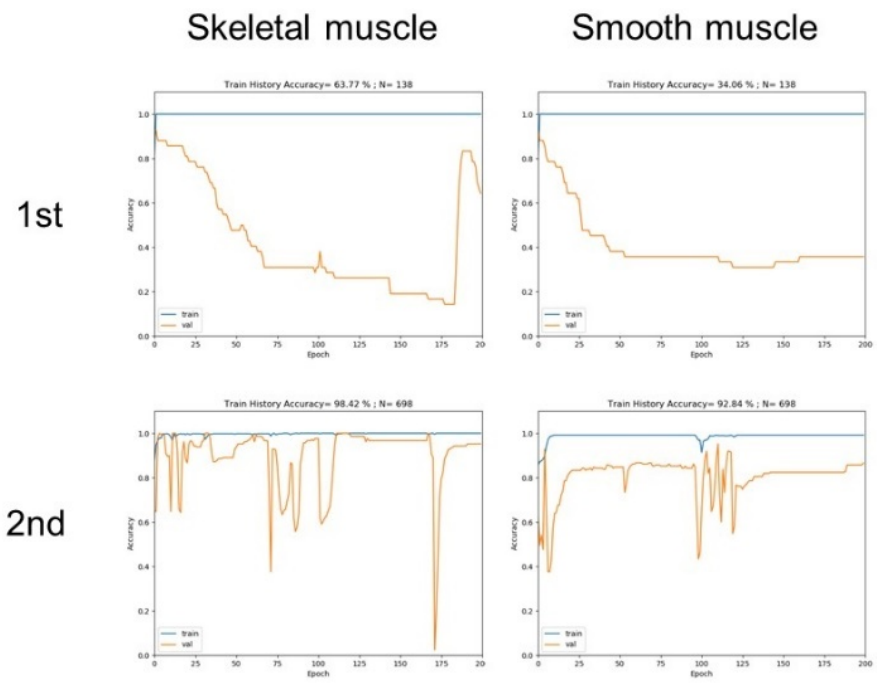


Fig 2b. Fig 2. Representative learning curves of CNN Models of skeletal muscle and smooth muscle of two serial experiments. The right and wrong images (shown in Fig. 1) of one specific microstructure, as a training data set, were used to train CNNs to extract each microstructure) features and these features were automatically approved in the validation data sets. During training, the CNN parameters were optimized by the validation data sets. The training program was run for 200 epochs to check accuracy of the specific microstructure model. The learning curve displayed the CNN accuracy (Y axis) of the training (train) and validation (val) data vs. the epochs (X axis). Two serial experiments were conducted on different image numbers in the training set: 138 images in the first (1st) and 698 images in the second (2nd) serial experiments.

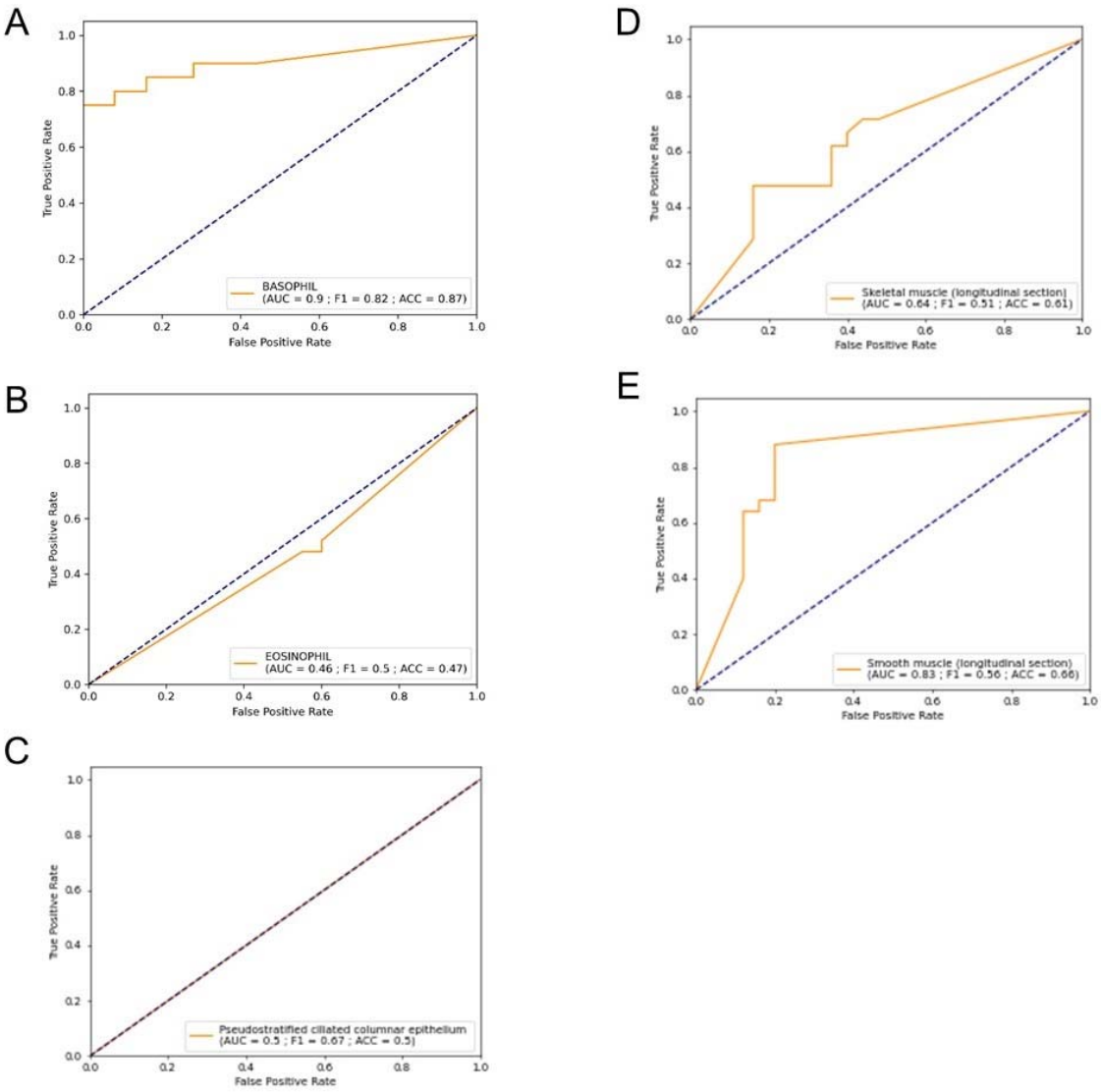


Fig 3. Representative ROC figures of accuracy performance of CNN models established by the first serial experiment. The CNN models were tested with additional test-sets of right and wrong images. The performance of the CNN models to detect microscopic structures was quantified by F1 measure, accuracy and the area under the curve (AUC) of receiver operating characteristic (ROC) and accuracy performance of CNN models was displayed as ROC figures. A: basophil; B: eosinophil; C: PSCCE; D: skeletal muscle; E: smooth muscle.

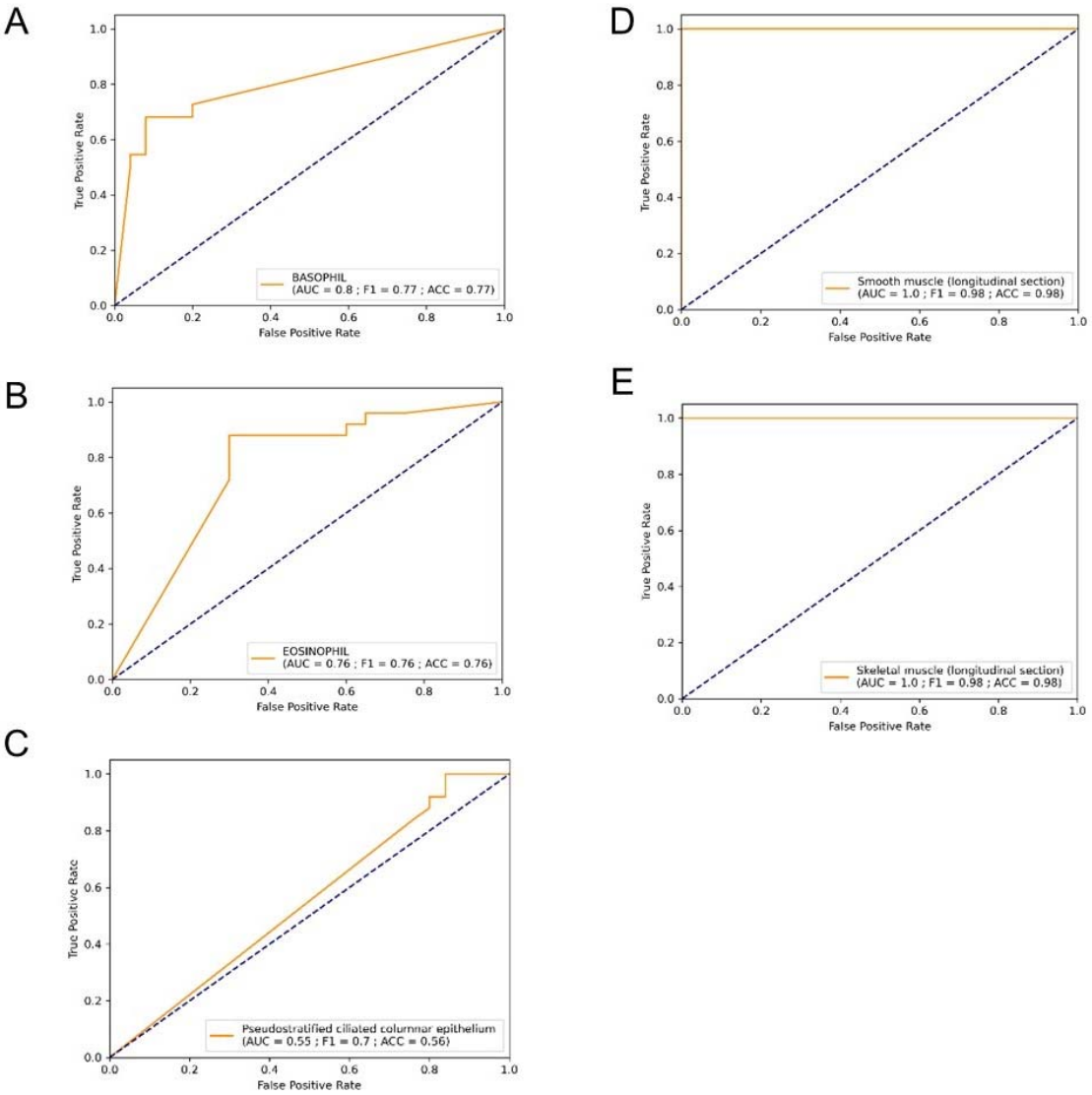


Fig 4. Representative ROC figure of accuracy performance of CNN models established by the second serial experiment. The CNN models were tested with additional test-sets of right and wrong images. The performance of the CNN models to detect microscopic structures was quantified by F1 measure, accuracy and the area under the curve (AUC) of receiver operating characteristic (ROC) and accuracy performance of CNN models was displayed as ROC figures. A: basophil; B: eosinophil; C: PSCCE; D: skeletal muscle; E: smooth muscle.

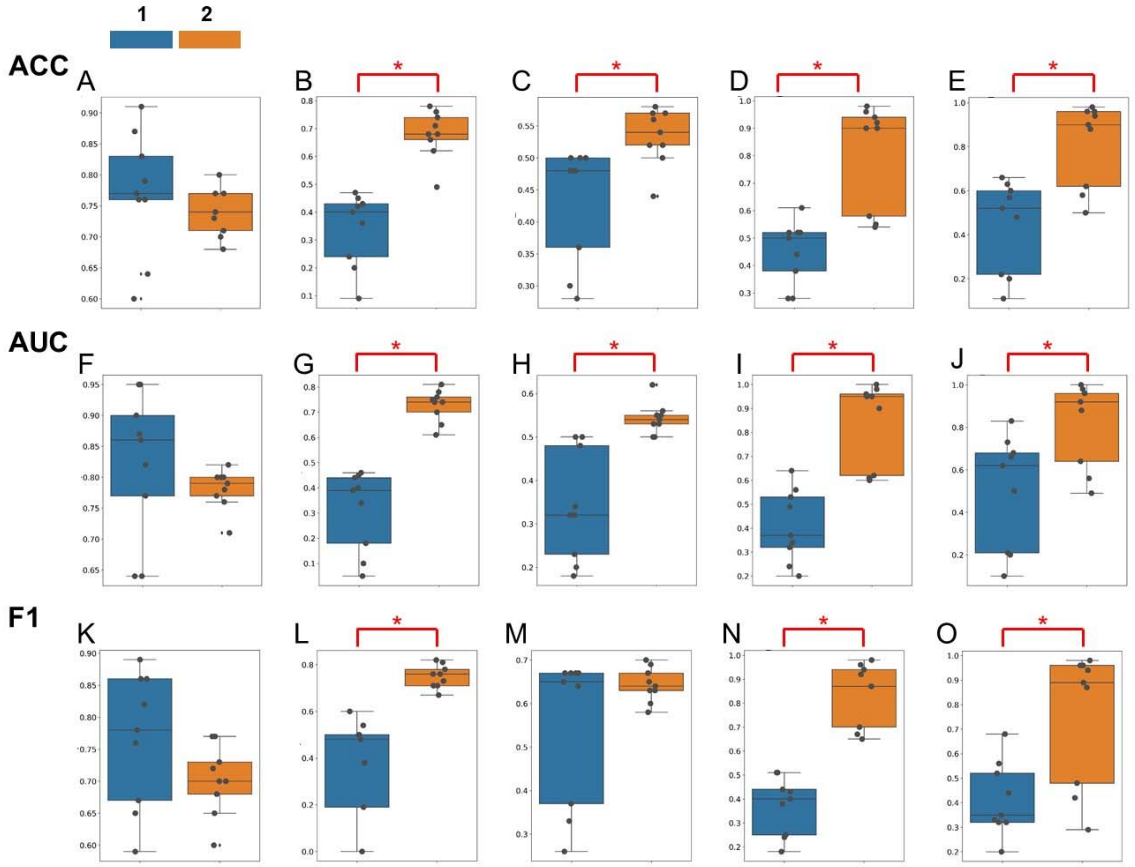
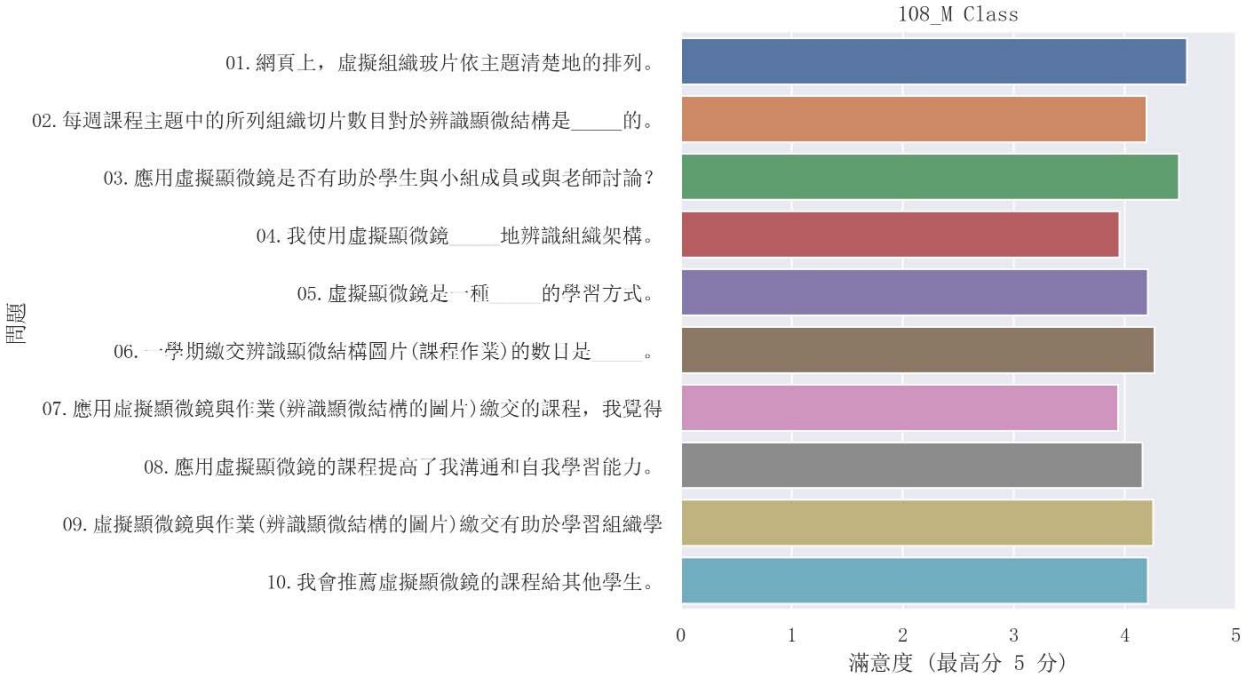


Fig 5. Performance assessment of CNN models between two serial experiments. The performance of the CNN models to detect microscopic structures were tested by F1 measure, accuracy and AUC between two serial experiments: the first (1) and the second (2) serial experiments. A, F, K: basophil; B, G, L: eosinophil; C, H, M: PSCCE; D, I, N: skeletal muscle; E, J, O: smooth muscle.

Table 1 Student evaluation of both virtual microscopy and supplemental assignments of submitting microscopic images 2-1



6. 建議與省思(Recommendations and Reflections)

The drawing picture of microscopic structures as the student assignment is a traditional way to weekly check student's performance by teachers. Drawing pictures can be replaced by submitting microscopic images when VM is implemented in histology laboratory classes. Our present results demonstrated that grading tasks of student microscopic structures can be automated and standardized with CNN. The correct images would automatically be excluded without expelling any images with the correct microstructures during image detection by successful CNN models. Then teachers check the images marked with wrong images, whether correct or not. Thus, the grading accuracy of student's images can be improved by the introduction of CNN. When student microscopic images can be identified by CNN for detection of microscopic structures, student performance's assessment can be carried out by CNN. Therefore, teachers have more time to keep track of student learning. The combination of VM and CNN offers an opportunity to improve histology teaching and learning. Time-consuming grading tasks of microscopic structure assignments by teachers can be automated and standardized with CNN, a form of AI.

An ongoing pandemic of coronavirus disease 2019 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is also known as COVID-19. During COVID-19 pandemic, online learning resources and off-campus self-active learning are the alternative for on-campus education. The virtual slide web site at KMU is available to the public via the internet at any place any place. Indeed, students can learn and study histology on-line, self-learning is no longer restricted by tissue slices on glass slides and light microscopes which are inaccessible when the school is closed.

Combination of VM and detection of microstructures by CNN can be used for on-line learning and alternative off-campus learning for histology laboratory teaching and learning during an ongoing pandemic of COVID-19.

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三. 附件(Appendix)

Appendix Table 1 The convolutional neural network layers of both first and second serial experiments for automatic detection of microscopic structures

Layer (type)	Output Shape	Param #
conv2d_1 (Conv2D)	(None, 360, 360, 4)	52
activation_1 (Activation)	(None, 360, 360, 4)	0
batch_normalization_1 (Batch	(None, 360, 360, 4)	16
conv2d_2 (Conv2D)	(None, 360, 360, 4)	148
activation_2 (Activation)	(None, 360, 360, 4)	0
batch_normalization_2 (Batch	(None, 360, 360, 4)	16
max_pooling2d_1 (MaxPooling2	(None, 180, 180, 4)	0
dropout_1 (Dropout)	(None, 180, 180, 4)	0
conv2d_3 (Conv2D)	(None, 180, 180, 4)	68
activation_3 (Activation)	(None, 180, 180, 4)	0
batch_normalization_3 (Batch	(None, 180, 180, 4)	16
max_pooling2d_2 (MaxPooling2	(None, 90, 90, 4)	0
dropout_2 (Dropout)	(None, 90, 90, 4)	0
Flatten_1	(None, 32400)	0
Dense_1	(None, 1)	32401

Total params: 32,717
Trainable params: 32,693
Non-trainable params: 24

Appendix Table 2 A questionnaire: Student perception about histology practical (laboratory) classes after implementing of both virtual microscope and supplemental assignments of microscopic structures

應用虛擬顯微鏡與繳交作業(辨識顯微結構的圖片)進行組織學實驗課的學生看法

同學好：

為了解課程的教學效果與缺失，用於改善教學，請同學表達你個人的意見。本問卷採不記名方式填寫。我們保證你的意見絕不會影響本科的成績。謝謝你的合作!

1. 網頁上，虛擬組織玻片依主題清楚地的排列？(A) 非常同意 (B) 同意 (C) 普 通 (D) 不同意 (E) 非常不同意

2. 每週課程主題中的所列組織切片數目對於辨識顯微結構是 ___ 的。(A) 非常足夠 (B) 足夠 (C) 普 通 (D) 不足夠 (E) 非常不足夠

3. 應用虛擬顯微鏡是否有助於學生與小組成員或與老師討論？ (A) 非常同意 (B) 同意 (C) 普 通 (D) 不同意 (E) 非常不同意

4. 我使用虛擬顯微鏡 ____ 地辨識組織架構。(A) 非常容易 (B) 容易 (C) 普 通 (D) 不容易 (E) 非常不容易

5. 虛擬顯微鏡是一種 ____ 的學習方式。(A) 非常有效 (B) 有效 (C) 普 通 (D) 沒有效 (E) 非常沒有效

6. 一學期繳交辨識顯微結構圖片(課程作業)的數目是 ____ 。(A) 非常足夠 (B) 足夠 (C) 普 通 (D) 不足夠 (E) 非常不足夠

7. 應用虛擬顯微鏡與作業(辨識顯微結構的圖片)繳交的課程，我覺得？。(A) 非常有興趣 (B) 有興趣 (C) 普 通 (D) 沒有興趣 (E) 非常沒有興趣

8. 應用虛擬顯微鏡的課程提高了我溝通和自我學習能力？(A) 非常同意 (B) 同意 (C) 普通 (D) 不同意 (E) 非常不同意

9. 虛擬顯微鏡與作業(辨識顯微結構的圖片)繳交有助於學習組織學？(A) 非常同意 (B) 同意 (C) 普 通 (D) 不同意 (E) 非常不同意

10. 我會推薦虛擬顯微鏡的課程給其他學生？(A) 非常同意 (B) 同意 (C) 普 通 (D) 不同意 (E) 非常不同意