Learning and Evaluating the Overlapping Role of Physics and Physiology in Perceiving Contrast and Resolution in Microscopy

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Abstract

Introduction:

Contrast and resolution have been fascinating to the students, as both interweave physics and physiology. Both these entities can be studied beyond the limits of the textbook on hands-on basis.

Objective

Determining contrast and resolution through human perception and technological tools for their quantification and estimation of the degree of overlap between the two disciplines.

Materials & Methods

An opaque repository for compound microscope with a mobile holder was devised to capture the images of Leishman stained smear. Another receptacle was fabricated to hold different coloured filters against a light bulb, which projected on the microscope mirror through a connecting pipe. The images with a different sequence of filters were subjected to analysis by human eye and also by digital image processing techniques.

Results

Perception of contrast and resolution through human eye and digital processing shows overlapping of 82.57% for contrast and 76.40% for resolution. Furthermore, white colour light was preferred for both contrast and resolution independently as well as combined, with propensity for contrast above resolution.

Conclusion

By comparing the effect of different filters on contrast and resolution, the integrated role of physics and physiology can be studied. Simultaneously, the subjective selection of resolution or contrast by using low-cost filters can be an affordable approach to upgrade the microscope.

1. Introduction

1.1. Objectives and overview:

Simple compound microscope has been an affordable apparatus used in laboratories across the globe. Learning the concepts of resolution and contrast, though essential, are difficult to demonstrate. With the dawn of digital teaching; cyber learning and e-mentoring, it would be fun learning these concepts in the laboratories as hands-on [1,2]. Simple manipulations in the simple compound microscope like low-cost filters can be used for imparting different contrast and resolution. The effect of these filters can be assessed by capturing the images digitally and analyzing them. This will not only help in learning but will simultaneously help in checking the efficiency of these filters for imparting subjective selection of resolution or contrast.

1.2. Background:

Though the microscope has now evolved tremendously, the basic light microscope with its credibility for ease of operation and affordability still stays as the mainstay in routine diagnostic purposes, research activities, and classrooms across the world. Microscope is an intricate amalgamation of physics, where comprehensive understanding of Optics can elucidate the concept of magnification and resolution.

Where *resolution is defined as "The ability to distinguish two objects or two points from each other even when they are lying too close to each other", Magnification is simply "Enlargement of the appearance of an object"* [3].

The brain perceives the details in an image by trading between contrast and resolution [4]. Where *contrast is "the difference in luminescence or colour that makes an object distinguishable"* [5]. Despite of this bargain that the brain deals with, there is out-and-out failure to perceive a clear image, the rationale for which can be the predetermined make of the microscope or failure to achieve practicable modification due to lethargy of the end-user. Therefore, it is necessary to circumvent these impediments to have a clearer image. These in our view can be achieved by economical modifications in microscope, which can also help in learning, as it involves problem solving, imitation, and engagement in authentic activities [1].

2. Methods

2.1. Equipment and Supplies

1. Microscope – a 'Simple compound microscope', which employs an external source of light for illumination.
2. Specimens- Stained thin blood smear and USAF target. The USAF target can be obtained from the physics department of your university or can be purchased.
3. Dark box – An opaque box with an approximate size of 70×45×40 cms with a lid; Box-A. It can be fabricated from any available material and can be either painted black or covered with black sheets of paper, serving the purpose of a dark room that intercepts the problems of ambient light while taking a photomicrograph [6].
4. Mobile & its adaptor for microscope –suitable to fit the mobile phone over the eyepiece to capture images from the microscope.
5. Light source- another lidded box measuring approximately around 25×25×25 cms; Box "B", having a holder to hold light source.
6. Opaque pipe- having an approximate length of 30 cms and an internal diameter complimenting the size of reflecting mirror of the microscope; approx. 5 cms.
7. Low-cost filter- Any filter which fits in Box-B that sieves the light from the source to the mirror.
8. Software for image analysis- any available software for image analysis (such as Matlab, Python, and Scilab)

In this study, our entire setup has been made with the materials that were economically feasible to us, however, any material can be used in lieu of. Our materials include a Monocular compound microscope (Bausch & Lomb Optical. Co Rochester, N.Y. USA. CL series with 100X Wenzel ptic wetzlar w-Germany 100:1 Oel NA 1.30), Leishman-stained blood smear, black Styrofoam boxes as Box A&B, Gosky cell phone adaptor, Samsung Galaxy Note 8 (SM-N950F in the pro mode of the camera), Panasonic LED bulb (Kiglo Omni, cool day, 1104 lm, Life 25000* BH, 12W, 6500K, 220-240V~50Hz, P.F. ³0.9 ta 45°C), dark PVC pipe, coloured gelatine paper (Blue, Green & Red), Matlab software (MATLAB-Matrix Laboratory; 2019b).

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the 'Institutional Ethics Committee for Human Research (IECHR) Medical College & SSG Hospital, Baroda' of University 'The Maharaja Sayajirao University of Baroda' Vadodara, Gujarat. (Date.16/05/2018/No. ECR/85/Inst/GJ/2013/RR-16).

2.2. Instructions

2.2.1. Assembly

With reference to Online Resource-I

1. On a flat surface (lab platform/table) Box A was positioned with breath on the floor and lid facing towards the performer. Box-B was placed adjacent to Box-A.
2. The microscope with mobile adaptor mounted on the eyepiece was placed in Box-A, with a mirror facing the side adjacent to Box-B.
3. A circumscribed circle with a diameter corresponding to that of the pipe was carved in the wall of the Box-A, at the level of the reflecting mirror of a microscope.
4. A circle akin to and facing Box-A was made in Box-B.
5. Connect both the boxes with the pipe.
6. A structure to hold different filters was fixed in Box-B between the light source and pipe inlet so that the light can be directed from the source to the mirror through the filter.

2.2.2. Procedure

1. The bulb in Box-B would be switched on, and the lid would be placed on it every time after a change in filter.
2. In the microscope fixed in Box-A, a stained smear slide would then be brought into focus under an oil immersion lens. Taking a cue from the setup made by Maude RJ et.al & Desai NJ et.al, once the cells were clearly visible, a mobile was mounted on the adaptor and its position and settings were adjusted so that it can capture the image of the field on voice command after putting a lid on Box-A [7] [8].
3. For the focused field, different coloured filters were fixed in the designated frame in the Box-B one after the other and their respective images were captured (Smear Images; Set-1).
4. Similarly, three more fields were focused, and images were captured.
5. Later, following the same sequence of coloured filters (as mentioned in point 3 above), images were captured using USAF as a specimen (USAF Images; Set-2).

2.3. Evaluation of the captured images

2.3.1. Human perception analysis

Set-1 images were clubbed together in a single sheet as in Online Resource-II where, four different coloured images of a single field were labeled as coloured images 1, 2, 3 & 4 of approx.8 cms diameter each on a single sheet and were printed. The sequence of the images for each field was changed to prevent habituation by participants partaking in step 1.

Step 1- Participant's response

A pre-structured questionnaire as shown in Online Resource-III was given to the participants, who were the routine users of a microscope. The participants had to arrange the Set-1 images from best to worst as per the questions. The printed images were accessed through a hollow eyepiece held at arm's length, giving a realistic experience of viewing through a microscope.

The data collected by step 1 was used to assess human perception in microscopy for contrast and resolution individually as well as both combined.

2.3.2. Digital analysis

Using a computer software, MATLAB (2019b); Set-1 & Set-2 images were cropped and converted to greyscale (black and white) images for an interested portion of an image. These images were analyzed by taking intensity profiles. These intensity profiles provide insight into how the intensity of the incident beam changes upon passing through the specimen, which in turn helps in defining the contrast and resolution of the system. Since, the sequences so derived are based on the merits of Physics, they are considered as a gold standard, ideal sequences for contrast & resolution.

Step 2- Contrast analysis

The Set-1 images were analyzed, as per Online Resource-IV. The derived ideal contrast sequence is summarised in table 1. The sequence was derived using the difference between the highest intensity pixel and lowest pixel. Since a sequence of images for each field had been different, the contrast sequence of each field had been derived separately, thus simultaneously circumventing the complicated task of sorting data. However, if a student wants, they can club the results of contrast from all the fields, to derive a single contrast sequence to proceed.

Table 1. Ideal contrast sequence.

	Field-I	Field-II	Field-III	Field-IV
IDEAL SEQ-CONTRAST	GWRB	WGBR	WGBR	WGRB

G=Green, W=White, R=Red & B=Blue coloured images, where it is written as best to worst. Example. For field-I G>W>R>B.

Step 3- Resolution analysis

To proceed with, let's first understand the resolution and its type from digital analysis point of view. Resolution is characterized by the finest detail that can pass through the system without being distorted. Where, "system" is the medium that connects the optical signal to the electronic output signal coming from the detector. Furthermore, the word "system" can be categorized into three subsections [9]; a) the free space medium where the optical signal propagates and the associated resolution is ***diffraction resolution*** b) when the signal is detected by a CCD camera, the spatial information is again distorted since the pixels of the CCD have a finite and nonzero size and the associated resolution is ***geometrical resolution*** [10] c) after the capture the optical signal is converted into an electronic signal and hence the quality of the detector such as its sensitivity, dynamic range, shot-noise level, read out noise, and other noises related to generation/recombination processes in the detector play a very crucial role. These noises further affect the resolution, and the associated resolution is ***noise equivalent resolution*** [11].

To add, according to Abbe [9], the diffraction limitation of spatial resolution in the camera plane can be expressed as

$$\delta_{xDIF} \approx 1.22\lambda F_{\#}$$

Where λ is the wavelength and *F#* is the F number of the imaging system.

This spatial resolution is related to the size of the aperture since

$$F_{\#} = \frac{F}{D}$$

Where, D is the diameter of the imaging lens.

While, Toraldo Di Francia [12] said that if one only wants to know the lateral distance Δ*x* of two stars, there is no diffraction limit on the accuracy of Δ*x*.

Now, proceeding with the availability of our resources, as done for contrast analysis, similarly set 2 images were analyzed for ideal resolution sequence as shown in Online Resource-V, and it is summarised in Table no.2. Since the magnification of the modified system was not known, the initial analysis of the USAF target images helped in deciding the magnification of the system. The smallest value of the difference in coordinates of pixel obtained for the smallest structure (Thorlabs R1DS1N: 6th element of the 7th Group; 2.2 μm) in any image among the images obtained by the combination of filters was considered for magnification. The sequence for resolution was obtained based on the value nearest to the magnification obtained above (i.e., with least scattering of light on the sensor).

Table 2. Ideal resolution sequence.

	Field-I	Field-II	Field-III	Field-IV
IDEAL SEQ-RESOLUTION	BWGR	BWGR	BWGR	BWGR

G=Green, W=White, R=Red & B=Blue coloured images, where it is written as best to worst. Example. For field-I G>W>R>B.

2.4. Further processing of data (as in online resource-VI)

1. A spreadsheet from the data derived from Step-1was prepared, and the frequency of each sequence was counted.

2. The nomenclature of sequences was then changed as per the ideal sequence derived from Step-2 and Step-3. To be labeled as 'revised sequence'. E.g., if in a field 1=Blue colour, 2=Green colour, 3=White colour, and 4=Red colour, and a selected sequence of a participant for contrast is 2314. And if the derived ideal sequence is Blue>Green>White>Red, i.e., 4321. Then, the participant's selected sequence for contrast, which was 2314, would be changed as per this ideal sequence and is labeled as revised sequence 3241. As the colour sequence in each field has been shifted to avoid habituation, having a new nomenclature would invariably provide with the best response as number 4 and the worst as number 1, thus aiding the scoring of sequences by using just a single formula.

3. Now as per the preference of selection, each response had been given a score as per the shift in its position from the ideal sequence as in Table no.3.

Table 3. Response Score.

If, 1st choice of the revised sequence =	4			
Then, shift in position of response from ideal position is	1st	2nd	3rd	4th
Score will be	4	3	2	1
If, 2nd choice of the revised sequence =	3			
Then, shift in position of response from ideal position is	1st	2nd	3rd	4th
Score will be	3	4	3	2
If, 3rd choice of the revised sequence =	2			
Then, shift in position of response from ideal position is	1st	2nd	3rd	4th
Score will be	2	3	4	3
If, 4th choice of the revised sequence =	1			
Then, shift in position of response from ideal position is	1st	2nd	3rd	4th
Score will be	1	2	3	4

Hence, the maximum score would be 16 and minimum would be 8.

4. The individual response of a revised sequence was scored, summed up, and multiplied with the frequency to obtain the total score.

5. The total score of individual revised sequences was then added to obtain the final score, and its percentage was obtained with respect to the maximum score. E.g., if the final score of the 244 participants is 3112, then the percentage matching would be (3112/244×16) ×100=79.71%

6. Similarly, to obtain the preferred choice of colour as the first choice for matching of contrast and resolution between the digitally analyzed image and perceived image, the maximum score was considered as 4 and minimum score as 1.

3. Results

Though it is a well-accepted fact that humans prefer contrast to resolution [13], it would be exciting to learn the same practically. Through this study, the degree of overlap between physics and physiology while perceiving contrast and resolution can be well understood. The actual value of contrast in an image can be derived, but the value of the limit of resolution cannot be derived, as the study pivots around the images captured by one system. However, the resolution obtained from different images in different light/filters can be compared. The results provide a better perspective to our questions;

Does the perception for contrast and resolution for different colours in microscope go parallel with physics?

Table 4. Percentage matching between human perception and digitally analyzed photomicrograph for contrast and resolution. (% matching for contrast and resolution)

	% Matching for Contrast (According to Q1)	% Matching for Resolution (According to Q2)	% Matching for Contrast & Resolution (According to Q3)	
			% Matching for Contrast -Q3	% Matching for Resolution -Q3
Field-I	79.71	76.33	78.12	74.85
Field-II	84.67	81.94	83.28	79.99
Field-III	88.48	76.03	86.34	75.82
Field-IV	77.42	71.30	76.75	71.32
Average	82.57	76.40	81.12	75.49

According to questionnaire, Q1 is for ease of cell identification; Q2 is for sharpness of cell border; Q3 is for identification and sharpness combined.

Table no.4 suggests, that perception of contrast and resolution goes parallel with Physics, 82.57% for contrast and 76.40% for resolution. While, from the average value of Q3, participants opt contrast against resolution.

For matching the contrast and resolution between the ideal and perceived image, which colour image is preferred as first choice?

Table 5. Percentage matching between human perception and digitally analyzed photomicrograph for preferred choice of colour for contrast only.

	For Q1. Ease of identification of cells.			
	% Contrast matching for blue colour Image	% Contrast matching for white colour image	% Contrast matching for red colour image	% Contrast matching for green colour image
Field-I	1.33	42.73	1.64	34.43
Field-II	7.41	23.05	0.10	46.30
Field-III	5.14	58.02	0.10	23.46
Field-IV	3.19	59.26	5.76	12.35
Average	4.27	45.76	1.90	29.13

From table no.5, percentage contrast matching suggests white, was the preferred colour of light followed by green, blue, and red for Q1 in all the fields

Table 6. Percentage matching between human perception and digitally analyzed photomicrograph for preferred choice of colour for resolution only.

	For Q2. Sharpness of the borders of cells.			
	% Resolution matching for blue colour Image	% Resolution matching for white colour image	% Resolution matching for red colour image	% Resolution matching for green colour image
Field-I	18.85	32.58	0.82	17.21
Field-II	37.45	19.75	0.10	17.90
Field-III	15.23	34.26	0.72	18.11
Field-IV	13.17	35.80	4.94	9.67
Average	21.17	30.60	1.65	15.72

Similarly, as shown in table no.6, percentage resolution matching for Q2 in all the fields, the preferred colour for light was white followed by blue, green, and red.

Table 7. Percentage matching between human perception and digitally analyzed photomicrograph for preferred choice of colour for contrast & resolution, both.

	For Q3. On their overall view. (i.e., considering both ease of identification and sharpness of borders both)							
	% Contrast matching for blue colour Image	% Contrast matching for white colour image	% Contrast matching for red colour image	% Contrast matching for green colour image	% Resolution matching for blue colour Image	% Resolution matching for white colour image	% Resolution matching for red colour image	% Resolution matching for green colour image
Field-I	1.74	35.66	0.82	43.85	6.97	35.66	0.20	22.13
Field-II	11.93	27.16	0.10	36.42	23.87	20.37	0.10	24.28
Field-III	4.12	57.20	0.31	25.00	8.23	42.90	0.31	16.67
Field-IV	2.37	58.02	6.58	14.51	9.47	43.52	3.29	9.67
Average	5.04	44.51	1.95	29.94	12.13	35.61	0.98	18.19

While as in table no.7, percentage matching for both contrast and resolution concurrently for question 3 in all the fields were white-coloured light above the green, blue, and red consecutively. This also suggests that humans opt for contrast over resolution.

4. Discussion

The current and the futuristic scenario for studying basic science has evolved to virtual microscopy, thus providing an immense aid in easing down the process of learning [14][15]. Despite of this evolution, experiments related to understanding of resolution and contrast are difficult to materialise. Moreover, in reality, while focusing an object under a microscope either contrast or resolution is achieved at the cost of other [4]. In this study, we tried to quantify and learn the bargain between contrast and resolution by the means of hands-on experiment. Upon reminiscing the background of our study, many questions would emerge in our minds as:

Q: On which of the entity does the quality of the image formed in the microscope depend: Resolution or Magnification?

A: Pondering on the answer, we often mistake magnification and resolution as one, though they are quite different as mentioned above. The resolution can be computed using the given formula.

Resolution (r) = 1.22λ/ [NA (obj) + NA (cond)] (Equation-1)

Here, *λ* is the wavelength of light that influences the resolution as a shorter wavelength provides greater resolution and vice versa. *NA (obj)* equals the objective numerical aperture, and *NA (cond)* is the condenser numerical aperture. However, several similar equations have been derived which show a relationship between resolution, wavelength, and numerical aperture, differing by a multiplication factor [16].

Where the numerical aperture is the ability of the microscope lens to gather light and resolve the fine specimen detail. It depends upon the refractive index of the imaging medium (medium between the front lens of the objective and specimen) and one-half angular aperture of the objective. Therefore, the higher the refractive index of the medium, the higher would be the numerical aperture and consequently the resolution power. In addition, the correction for optical aberration has an impact on the numerical aperture of an objective. Higher the correction for chromatic and spherical aberrations larger is the numerical aperture for respective magnifications [17,18].

Furthermore, resolution cannot be defined without the reference of contrast with which it shares a reciprocal relationship. When viewing in the microscope, a specimen is imperceptible by the human eye, as the specimen itself has little or no contrast. Unlike resolution and magnification, it is unfeasible to derive contrast as it is multifactorial. These hurdles lead to the discovery and evolution of staining. An adequate contrast is a key to decipher the complete microscopic details of a magnified and well-resolved specimen. Hence, the elements influencing resolution are the type of specimen, coherence of illumination, degree of aberration correction, and other factors such as contrast-enhancing methodology either in the optical system of the microscope or in the specimen [19].

Summarizing it, magnification as a sole component of an optical system yields an enlarged image without delineation of minute structures so that the enlarged image is perceived by the human eye. However, resolution makes it more meaningful by adding minute details in it. Thus, we can interpret that for formation of a quality image at eyepiece, both magnification and resolution hold equal ground.

Q. Which sources of light are used in the microscope and why?

A: The visible spectrum ranges from 400–700 nm, of which the human eye is more sensitive to 550 nm, which corresponds to the green colour. So, microscopists prefer using the sources that emit this spectrum of light (400–700 nm, centered at about 500 nm) for bright field microscopy [19]. The most common example is the tungsten bulb. While other examples are mercury arc lamps, xenon arc lamps, metal halide lamps, LED's, etc. [20].

In addition, the NA inscribed on the objectives used for calculation of resolution is determined by the manufacturers keeping this wavelength in mind.

Q: How to derive magnification and resolution in a classroom?

A: Magnification power is determined by multiplying the magnification values of the objective and the eyepiece that are imprinted on the microscope.

Generally, resolution is estimated by measuring either the minimum resolvable distance between two adjacent structures in an image, or alternatively, it can be estimated from the intensity profile analysis such as simple full width-

half-maximum (FWHM) or more complex fitting [21] of sub-resolved sized structures. Techniques such as Fourier ring correlation (FRC) [22] and Fourier shell correlation (FSC) have been used for decades to estimate image resolution in electron cryomicroscopy [23]. FRC was also adopted in the field of optical nanoscopy to address the issues with the traditional resolution assessment methods [24–26]. However, this would require sophisticated designed tools or models, which take a lot of expertise and time. Hence, for a resource poor setups, the method used in this study would be the most feasible.

While crudely, resolution is derived using above equation-1 where the wavelength is taken as 550 nm and NA is taken from the imprinted value on the eyepiece as explained above.

Q. Why there is a preferential use of blue filters instead of green, even though human eyes are more adapted to green colour?

A: Incandescent tungsten-halogen lamps display colour shifts with fluctuation in light intensity. When the intensity of the lamp is low the spectrum of the light is more towards the longer wavelength of light while when the light intensity is high the spectrum is more towards the shorter wavelength. This can result in a significant shift of colour in the spectrum, which is ignored while visualizing the images through the microscope but is exasperating while capturing the micrographs. In micrographs, at lower intensities, the image has a more yellowish-orange background, while at higher intensities the image appears more natural [27]. When a blue filter is applied over the lamp, light with a longer wavelength is absorbed, giving a much cooler and natural image. Hereafter, it is not necessary to correct the temperature of the picture. In addition, as mentioned above, light with a lower wavelength increases resolution. On the above-mentioned grounds, some microscopes come with an inbuilt blue filter placed under the condenser.

At this point, it is worth mentioning here that various studies have depicted the hazards of blue light [28]. So, looking at the pros and cons of blue light, its use becomes quite subjective.

Q. Should we use a single monochrome light to obtain the best results in our microscope?

A: Practically yes but for perception by the human eye, we need contrast to identify the object of interest, so a polychrome source of light and staining the specimen is the option that we would have. While for image processing assisted with machine learning or deep learning algorithms a single light will be better, as it will avoid many optical aberrations [29 30].

Q. Why do we use a combination of Stain?

A: In the bright field microscope, a light source that emits visible spectrum is used. Hence, if the objects are colourful, they would be perceived well. Imagine watching a black and white television over a coloured one, which one would be more appealing and informative? It's just the same with microscopy, where we can better understand this concept by observing an unstained and a stained slide. If we talk about the present study, here the Leishman stain has methylene blue and eosin that stains the acidic and basic structures as per its strength; it imparts an overall shade of purple and violet on a slide. Hence, from a perceptive point of view, a single stain fails to impart enough contrast compared to two. Thus, to have better differentiation in a specimen concoction of stains is necessary [31].

Q. Can wrapping gelatin paper over the source of light improve the image quality?

A: The results suggest that the answer will depend upon an individual's preference to opt for either contrast or resolution, as the ideal sequence for contrast and resolution are variable, as shown in table no 1 & 2. Hence, the selection of filters is purely subjective. To add, since the properties of gelatin paper related to the amount of spectrum

of light that can pass through it or be blocked by it, is not known it cannot be relied upon for improving the image quality as far as the reproducibility of the results is concerned. So, we can say gelatin paper is just helping in changing the hue of an image, which can be used as subjective preference.

Q. Do the stain and the light used in a simple compound microscope provide the best resolution and contrast?

A: The results suggest that white light is best with Leishman stain, providing maximum contrast and resolution. The results distinctly indicates that there is considerable overlap for contrast and resolution and that humans have more tendencies for contrast, thus bargaining resolution.

As mentioned in the article above, we can either have resolution or contrast but not both. The hazard of blue light suggests avoiding blue light while maintaining contrast or increasing the contrast with minimal compromise of resolution. Hence, we propose there is a need for a stain that can give a green background while staining the specimen yellowish-white or any other contrasting colour. This is because after hazardous blue light shade, it is the green shade that provides maximum resolution and favours physiology of perception for green colour.

Since, many attempts made in past have had a great leap of success in increasing contrast and resolution (like phase contrast, fluorescence and all). The current study is only a small step in getting customized contrast and resolution in the existing usable simplest microscopes in many developing and least developed countries. It simultaneously suggests that a user can maintain good health of his eyes by avoiding prolonged and fatiguing exposure to the blue light.

Q. Is the setup of this study ideal? Are the results reliable?

A: No, neither the setup nor the results are completely ideal or reliable. While it can be said that the results and setups are good for half of the experiment, i.e., considering for the contrast the setup and results are very well reliable. While for resolution the method used is correct, but the setup is not the ideal one. Hence, though the setup is affordable and accessible, the offset is due to the use of low-cost filters and other optical components. Consequently, there is a need for standardization of setup considering the ideal setup protocols like for the illumination/intensity of light, capturing device and its filter (like CFA/IR cut) used, adjustment for condenser/iris/fine adjustment for perfect focusing (i.e., proper alignment of all the optical components) [3233]. Such standardization would give more reproducible results with different devices like CCDs or mobiles.

4.1. Evaluation of Student Work

The entire exercise would be contemplated as self-evaluation. The data derived would depend upon the quest of a student to seek answers to the questions in his mind. Based on results, the student can compare the outcome of different filters as per the subjective preference and also conclude if the filter used was apt or not.

4.2. Wider Educational Applications

On a broader base, it is a confluence of two fields, viz. the medical field whose sole focus is to visualize a cell by emphasizing contrast and the technocrats/field of optical science that exclusively prioritizes on ameliorating systems resolution. Hence, this would be the most apt way to have an integrated learning. As recommended by Kent J. Crippen in his book, this would help in collaborative problem solving in the learning environment by organic integration of two different fields of science [1].

4.3. Additional Experimentations

Q. Is further accretion of data possible in this study?

A: Certainly, the data can be amassed using different sources (tungsten incandescent bulb, monochrome LED, CFL), filters, specimen (ZN stain, Gram stain), and observing devices (mobiles, CCD).

Q. Is there any scope for ancillary studies/experiments that can ameliorate the simple compound microscope?

A: The boundless possibility of a simple compound microscope is what makes it more intriguing. It is the need of the hour to have an affordable and easily tuneable technology that can add more meaning to the existing one.

Yes, there are other additional possibilities for the experiment as below: -

 Rotating filters: to reduce the hazards of blue colour on the eyes, a rotating filter can be used between condenser and mirror. That is, a device comprising two or more coloured filters rotating at the desired rate can be designed that can fit between the condenser and reflecting mirror. Example: fitting the device with green and blue filters alternately. At a higher speed, the device imparts an overall cyan colour, which can be evaluated for its quantification for contrast and resolution.

 Converting simple microscope to anaglyph microscope: two slightly separate images can be produced at the eyepiece with the use of the above device with coloured filter; or coloured polarised filter; or directly using monochrome lights, which can rapidly on and off alternately. The degree of separation of images can be adjusted by adjustable prism/or non-colour corrected glass at the eyepiece. These distinct images will be displayed on a screen affixed at the eyepiece, which can be visualized using 3D glasses.

5. Conclusion

Learning the quantification of contrast and resolution from the physics and physiology point of view, though lengthy, is worthwhile, as our results found that they go parallel 82.57% for contrast and 76.40% for resolution. An interesting observation made was the human inclination towards contrast was 81.12%, compared to 75.49% for resolution. White light was the preferred choice by the majority of the participants to obtain maximum contrast and resolution in a simple compound microscope. Being the most integral apparatus for the field of medicine and research in developing and underdeveloped nations, learning to apply customized modification in a simple compound microscope in an affordable way is a necessity.

Declarations

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Conceptualisation: [Dr. Prashant Rajdeep]; Methodology: [Dr. Preeti Panchal], [Dr. Prashant Rajdeep], [Dr. Vismay Trivedi]; Formal analysis and investigation: [Dr. Prashant Rajdeep], [Dr. Vismay Trivedi], [Dr. Rinkesh Chaudhari], [Dr. Kinjal Parmar]; Writing - original draft preparation: [Dr. Prashant Rajdeep]; Writing - review and editing: [Dr. Prashant Rajdeep], [Dr. Lajja Patel], [Dr. Vismay Trivedi]; Funding acquisition: [Dr. Prashant Rajdeep]; Resources: [Dr. Prashant Rajdeep], [Dr. Lajja Patel], [Dr. Vismay Trivedi]; Supervision: [Dr. Prashant Rajdeep].

COMPETING INTERESTS

The author(s) declare no competing interests.

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CONSENT TO PARTICIPATE AND TO PUBLISH

Written informed consent was obtained from all individual participants included in the study.

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