Reinventing the Microscope in the Age of Digital Imaging

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Abstract

Human requirements place many constraints on compound microscopes making them complex and expensive instruments. By replacing human eyes with an image sensor a simpler design can be used. This paper considers the design of a microscope with an image sensor which optically magnifies an object 20 times using a single lens to achieve a real magnification of over 500 times. A single lens design is analyzed but found to be inadequate so a standard microscope objective is used.

Keywords: microscope, pollen, image sensor

1 Introduction

Pollen identification is important to agriculture, horticulture and healthcare, but is time consuming because the extraction and modification of pollen is done by manual preparation and analysis [1]. Some research has been done into automating the identification of pollen [2,3], based on the use of the texture features on images of pollen [4]. While this research shows promise, it does not address the problem of image acquisition. In current techniques, pollen slides are analysed under a microscope and a trained researcher will take 2-10 hours to analyse a slide[5].

Imaging pollen introduces some application specific constraints. Pollen analysed this study range in size from 20μm to 80μm. To be useful for texture based classification the images must contain as much useful texture information as possible. The finest texture on a pollen grain is much smaller than can be can be imaged using visual light, so it is necessary to settle for the image provided by magnifying the pollen to the limits of optical resolution, calculated using Raleigh's criterion and approximated to 900nm. To obtain visible images of objects of this size a compound microscope is conventionally required.

2 Analysis

The design of modern compound microscopes is constrained by its intended user, that is the human being. Humans place demands on the magnification, image quality, illumination levels and packaging of a microscope. In particular our eyes, with their integral lens, are not biologically designed to view microscopic images and therefore require optics to provide magnifications in the range 400x – 1000x in order to view pollen. To obtain magnification of this magnitude and preserve image quality requires complex optics, as simple optics designed to provide high magnification alone will exhibit severe image degradation due to lens aberrations.

A typical laboratory microscope suitable for viewing pollen has 8-15 lenses in the objective and 2-5 lenses in each eyepiece. Given that every lens added to the microscope adds expense, it is logical to seek a means of replacing our eyes with a technology that does not require such high magnification, and is more suitable for analysing and classifying microscopic objects. If the intention is to use digital image processing methods and a solid state camera to replace the human, then many lenses and features of the modern precision microscope can be dispensed with.

An image sensor, such as a CCD, is microscopic in nature. The size of an element, a pixel, on the sensor is similar in magnitude to the microscopic detail that is of interest. This substitution of a sensor for the human eye reduces the magnification required, and has two effects on the design of the optics:

1. Reduced magnification reduces the number of lenses required to enlarge the object

2. Reduced magnification reduces the number of lenses required to correct for aberrations introduced by high magnification.

Thus significant savings in optics, and therefore cost, can be made by removing from the microscope the optics that are required to meet the needs of human eyes. However this is not the limit of the possible simplification. If a microscope could be built using just a single lens it would vastly simplify the construction and so reduce the cost.

3 Feasibility of a Single Lens Microscope

The concept of a single lens microscope coupled to an image sensor has been implemented at low magnifications by Intel. The Intel QX3™ Toy Microscope has an integrated camera that captures images of specimens at 10×, 60× and 200× magnification. The optical magnification for the 200× magnification is performed by a single custom lens which magnifies 4x [6].

The QX3 has limitations which prevent it being used as a scientific instrument. Its highest magnification, nominally 200x, is inadequate for resolving the textural detail in pollen that is required for classification. Additionally the sensor used in image capture contains only 320×240 elements, which gives a restricted view of the scene.

In order to overcome these limitations the power of the optical system used in the QX3 would need to be increased. This would push the system into optical limitations such as aberrations and diffraction.

3.1 Resolution

The fundamental optical limitation is the resolution of the optical system. Under incoherent light this is given by:

$$d_{\min} = \frac{0.61\lambda}{n \sin \alpha} \tag{1}$$

where λ is the wavelength of radiation, n is the refractive index and α is the aperture angle of the lens [7].

To calculate this the fixed parameters of the system must be known. They are the lens focal length(f) and the sensor size(R_{img}), the latter represented as a maximum distance from the centre of the sensor to and edge, which is also the maximum extent of the lens needed for imaging. Figure 1 shows these parameters.

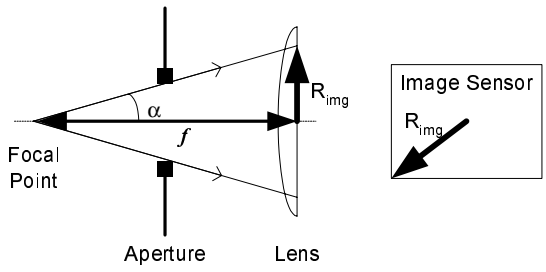

Figure 1: The parameters used for calculating α.

Using the largest unfiltered wavelength, λ=700nm, and a refractive index, n = 1.664[1], the resolution is calculated as $d_{\min}$=2.17μm.

It is not possible to produce perfect images. Image fidelity is limited by lens aberrations and ultimately, diffraction. However if the degradation cannot be resolved by the sensor or perceived by a human then the image can for practical purposes be considered to be perfect.

3.2 Aberrations

All spherical lenses produce aberrations, phenomena that degrade the image. The most obvious of these is chromatic aberration, where light of different wavelengths is refracted differently causing 'rainbow edges' in colour images, and blurring in monochromatic images. Using white light at 20 times magnification the blurring from chromatic aberrations will be in the order of 20μm. This means that two overlapping objects less than 40μm will blend with each other.

Spherical aberration is caused by different regions of the lens focusing light at different distance from the lens and causes general blurring to an image. Spherical aberration can be calculated using ray-tracing techniques such as those explained in [8]. There are two key factors that affect the spherical aberration:

1. The aperture size limits the maximum ray divergence from the object, limiting the divergence of meridional rays. Therefore the spherical aberration is reduced as the aperture is closed.

2. The refractive index of the lens determines the severity of the refraction at the surfaces. A smaller refractive index causes less divergence and therefore less spherical aberration.

3.3 Diffraction

Diffraction also limits the resolution. An aperture has been introduced into the system to control the illumination and spherical aberration. As the aperture is a limiting edge it is a barrier which light diffracts around. Using a single-dimension simplification, it can be shown that the spread of the central maximum of the diffraction emanating from a point within a slit has the following formula:

$$W = \frac{2L\lambda}{b} \tag{2}$$

where W is the width of the central maxima, L is the distance from the slit to the image, b is the slit width, and λ is the wavelength of the light[9].

[1] The lens with these parameters is A45-097 from Edmund Optics [11].

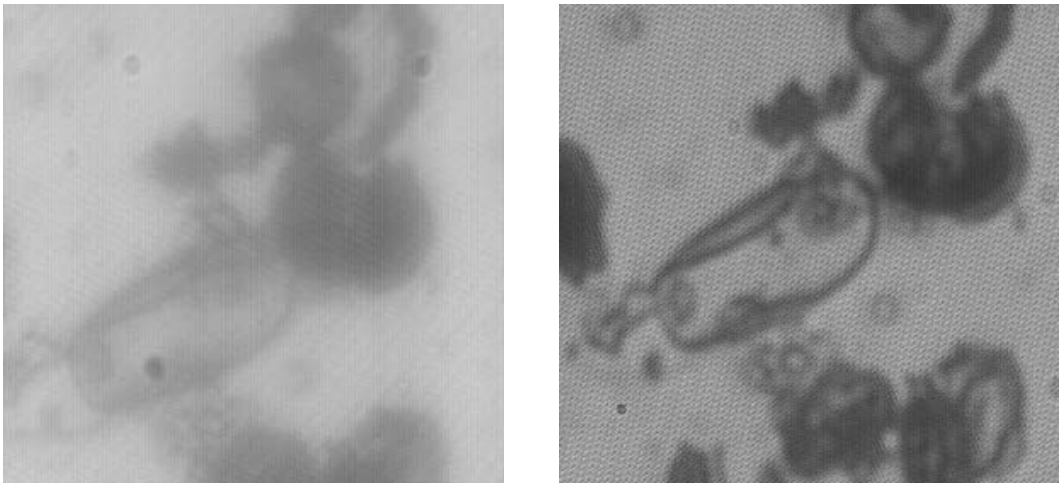

Figure 2: Effect of applying a narrowband filter to the light source.

3.4 Correcting Aberrations

Correction of chromatic aberration typically requires an achromatic doublet, which introduces a negative lens with a high refractive index. An alternative solution is to limit the wavelength of the source. During early experimentation a filter was applied consisting of a Near-IR Cut Filter on the camera transmitting $\lambda < 700$nm, and a red filter on the source transmitting $\lambda > 630$nm. Thus the illumination has a bandwidth of 70nm, compared to the bandwidth of white light at $\approx$ 320nm. The effect is to improve the detail in the image as shown in Figure 2.

This is an acceptable limitation for our application, and many image processing applications, where colour information is not used.

Correcting for spherical aberration requires that the aperture diameter be decreased. However it can be seen from equation 2 that decreasing the aperture (parameter *b*) increases diffraction. Thus we have a trade-off between aberration and diffraction.

Applying equation 2 shows that an aperture greater than 21mm is required to reduce the effect of diffraction to the desired value of 2μm. However this increase in aperture size increases the quantity of non-paraxial rays in the system and increases the magnitude of the aberrations.

In order to correct for spherical aberration the diameter of the point spread function (p.s.f.) should be at most 2.2μm. To achieve this an aperture of diameter 0.061mm is required. At this aperture diameter the effect of diffraction would be a p.s.f. with diameter 12mm or about the half the area of the imaging sensor. Additionally an aperture of this size would block out 99.9% of the image.

3.5 Conclusion

This trade-off between aberrations and diffraction in the optical system prevents achievement of the optimal resolution. Although the values here are for the case of a lens with *f*=25, a lens where the diameter of both the spherical aberration and beam spread is smaller than 2μm cannot be found by applying the ray tracing and beam spread equations to lenses with both shorter and longer focal lengths.

Therefore, a single lens microscope magnifying twenty times cannot provide the resolution required for imaging pollen.

4 Design of a Computer Microscope

4.1 Design Alternatives

There are two likely alternatives for the basic design of a computer microscope.

The first option, custom optics, allows greater design flexibility. Triplets, such as Cooke's Triplet[10], can adequately correct for all primary aberrations and chromatic aberration. However they require considerable optical and mechanical design.

The second option, a standard finite achromatic microscope objective, is less flexible than custom optics, but has known design parameters, is mechanically housed, and contains corrective optics for all primary aberrations. Importantly, the cost of a standard objective and the cost of three lenses plus housing is approximately equal[11].

As this is an integration application the standard microscope objective is the prudent and expedient option and has been selected.

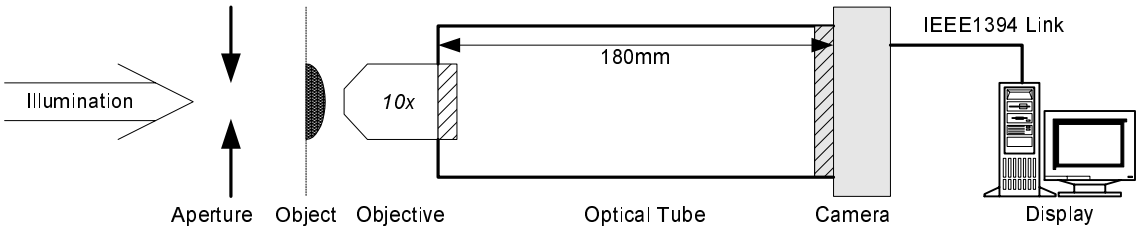

Figure 3: Design of a Computer Microscope to optically magnify 12×

4.2 Selected Design

The selected design for a computer microscope, shown in Figure 3, is vastly simpler than a conventional compound microscope. Most importantly the optical magnification has been greatly reduced. At lower magnifications illumination is not as critical as it is for higher magnifications. This removes the need for complex and expensive condenser optics and illumination train and allows a cheap source of illumination to be used, such as an incandescent light bulb.

This computer microscope is more flexible than a conventional compound microscope as it is not bulky or fixed in place, making it suitable for field work.

This computer microscope stands apart from existing entry level computer microscopes such as the QX3 by providing significantly higher magnification.

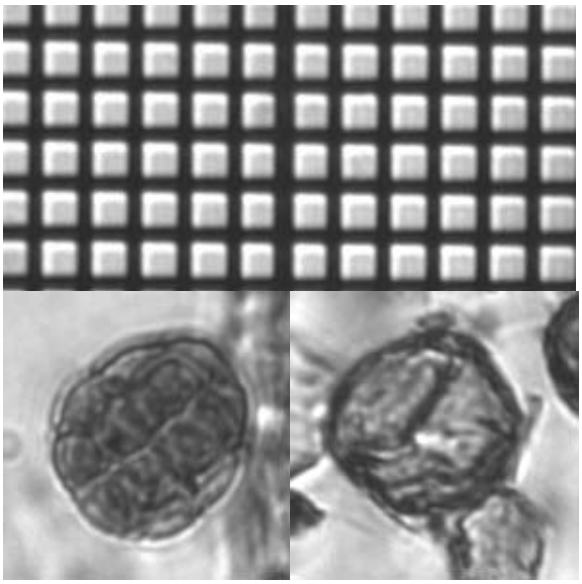

Figure 4: Image of a calibration grid and two pollen captured using the computer microscope at 12x optical magnification

5 Results

A standard 10× achromatic objective lens was used to acquire the images. By using different optical tube lengths magnifications in the range 10-22x we obtained. It was found that above about 12× increasing the magnification did not increase the resolution. Furthermore, the 12× images, displayed in Figure 4, contain pollen of about the right size and of sufficient detail to be classified using the existing classification algorithm.

The contrast of the images captured could be improved by correction and, expensive, illumination. However this degradation is not critical and can be improved in software by using contrast expansion algorithms.

6 Conclusion

If an optical microscope is not designed for direct human viewing, then the need for complicated optics is eliminated. This makes it possible to construct a simple microscope using only a standard microscope objective and a digital image sensor. As a consequence of the direct use of a solid state sensor having microscopic elements to image an object, the resulting microscope has lower optical magnification than a conventional microscope. Additionally the computer microscope does not need expensive illumination systems and can be positioned flexibly.

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