Image Quality and the Microscope Slide: A New Approach

CALAMAT®

CHAS MANGHAM*, MELANIE HUGHES, SARA CARNEIRO, MARTIN PRITCHARD, NICOLA EDWARDS
Calamat Ltd, Bramhall Moor Lane, Hazel Grove, SK7 5AA
*Corresponding Author

Introduction

In the field of light microscopy, the configuration of the microscope slide has not changed in over a century.

It is evident that there are optical shortcomings of the standard microscope slide, which impact on image quality. We redesigned the microscope slide with the goal of improving image resolution, contrast and flatness, and reducing variation between images.

The standard microscope slide configuration is pictured in Figure 1. Problems include:
i) **Composite** coverslip thickness equals coverslip mountant **plus** coverslip glass thicknesses. Mountant thickness may vary significantly. Deviation from optimal composite coverslip thickness introduces optical aberrations, especially spherical aberration[1], affecting image resolution.
ii) The standard, one-millimetre-thick, glass microscope slide introduces further optical aberrations, especially chromatic aberration.
iii) Standard microscope slide soda lime glass produced by the float-glass method, resulting in an undulating surface at the micron scale.

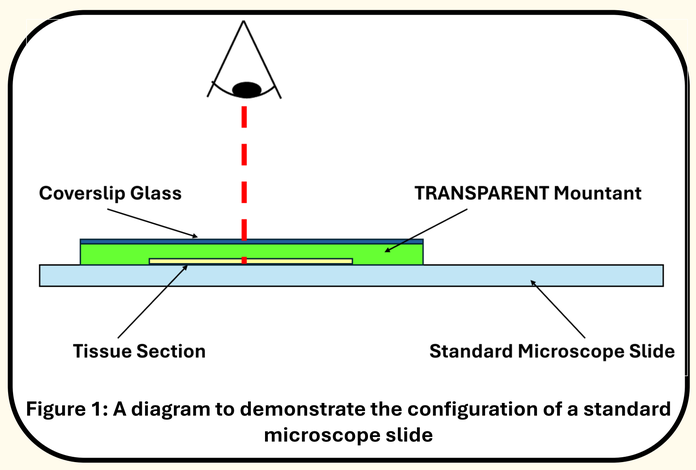
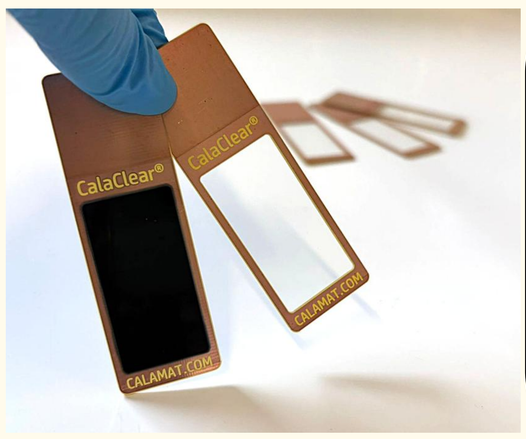


Figure 1: A diagram to demonstrate the configuration of a standard microscope slide

Methods

I) Re-design of the microscope slide:
The novel CalaClear® microscope slide configuration is shown in Figure 2.
i) The tissue is applied directly to the undersurface of the coverslip glass. The coverslip thickness is no longer composite and variable because the mountant no longer contributes.
ii) The removal of a one-millimetre-thick layer of soda lime glass with the aim of reducing chromatic aberration.
iii) The borosilicate coverslip is made by the draw glass method. Compared to the aforementioned float-glass method, this provides a significantly flatter surface with the aim of improving the focal plane.



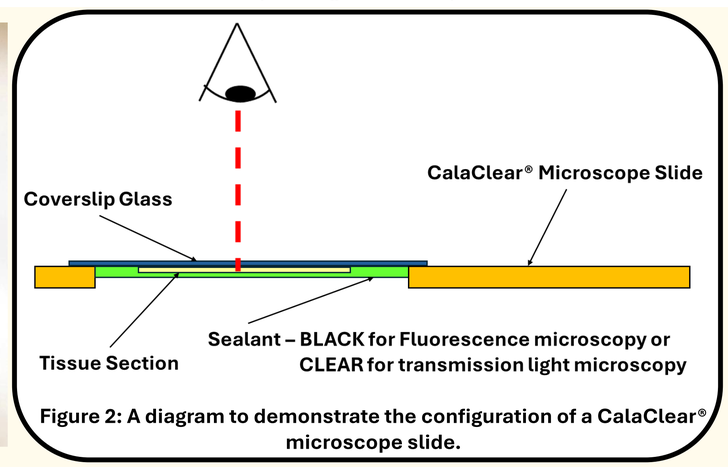


Figure 2: A diagram to demonstrate the configuration of a CalaClear® microscope slide.

An additional, and important, design feature is that different types of sealant can be applied onto the back of the tissue within the well. Clear sealant for transmission light microscopy or black/opaque sealant for fluorescence microscopy. The use of black sealant aims to reduce background fluorescence.

II) Evaluation of the novel microscope slide:
We present our re-designed slide's utility and performance for fluorescence microscopy; similar benefits are gained in transmission light microscopy.
An evaluation study was performed by comparing images obtained on tissue sections prepared using the CalaClear® microscope slide alongside the images obtained with the standard microscope slide.
Standard histology techniques were used to prepare formalin fixed, paraffin embedded tissue (FFPET) sections of a variety of tissues - lily pollen, lily stem, tumour, normal testis, and a normal sweat gland. Mammalian tissue was stained with fluorescent dyes. Plant tissue did not require staining due to its strong autofluorescence.

Epifluorescence microscopy was performed with the following equipment:
- Lily pollen grains - Nikon ECLIPSE Ti2-E inverted research microscope with an objective lens Plan APOCHROMAT 40x/0.95 numerical aperture (NA) and utilised a pE-4000 light source (CoolLED). Prime 95B 22m cMOS camera (Photometrics) used for image acquisition. Four channels were captured – brightfield, blue, green and red, with the images shown in the green channel of excitation wavelength 470nm. Image acquisition and settings were controlled through the NIS-elements AR software (Nikon).
- Tumour and normal testis - Olympus BX43 upright microscope. Tumour - objective lens Plan APOCHROMAT 100x oil-immersion/1.25 NA. Normal testes – objective lens UPlanFL N 20x/0.4 NA.
- Normal sweat glands - Olympus BX53 upright microscope. Objective lens Plan APOCHOMAT x40/0.7 NA.

InnoQuant fluorescence whole slide scanning was performed with the following equipment:
- Lily stem - InnoQuant[2] Image Acquisition & Image Processing. Four lasers (357nm, 488nm, 561nm, 640nm) and four photomultiplier detectors (PMTs), with seven filter positions per PMT. Resolution up to 0.5µm/pixel (approximate equivalence NA 0.4).

Results

The following images are representative of the microscope image quality evaluation, demonstrating each of the improved parameters achieved by using the CalaClear® microscope slide with black sealant for fluorescence microscopy.

i) IMPROVED IMAGE RESOLUTION (Figures 3 and 4).

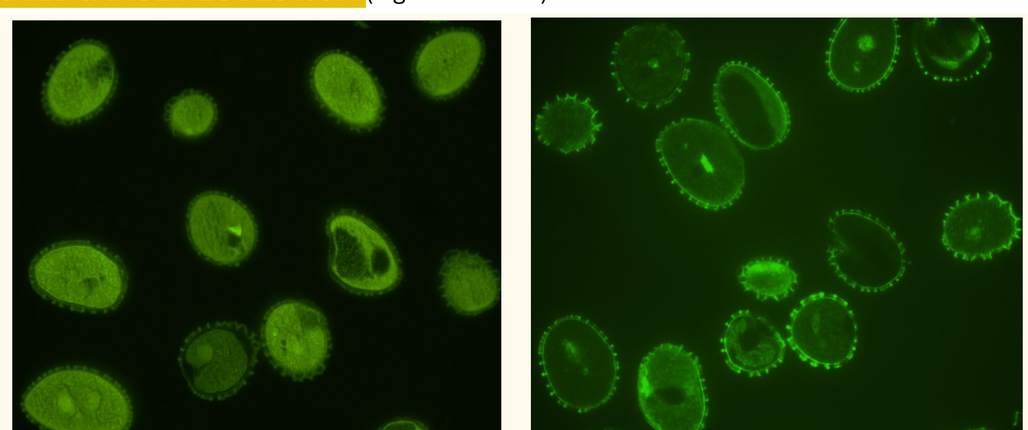


Figure 3: Images of lily pollen prepared on a standard microscope slide (left), and prepared on a CalaClear® microscope slide with black sealant (right). Nikon ECLIPSE Ti2-E inverted, original magnification x40, NA 0.95.

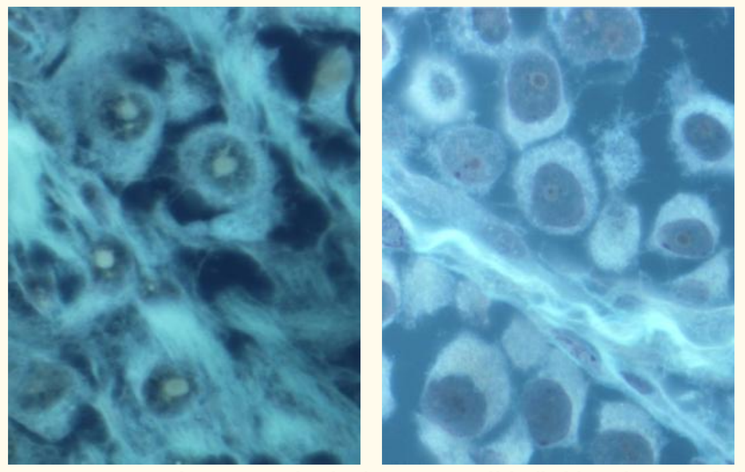


Figure 4: Images of a tumour prepared on a standard microscope slide (left) and on the CalaClear® microscope slide with clear sealant (right). Olympus BX43, original magnification x100, oil-immersion, NA 1.25.

ii) IMPROVED IMAGE CONTRAST (Figure 5).

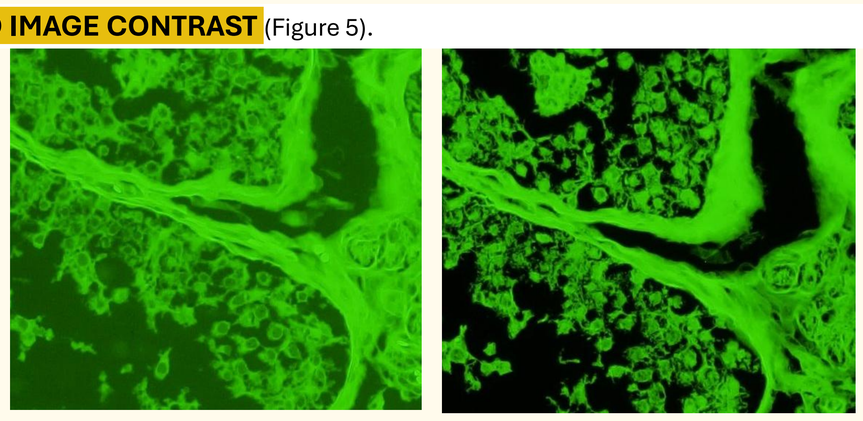


Figure 5: Images of normal testis tissue prepared on a standard microscope slide (left) and on the CalaClear® microscope slide with black sealant (right). Olympus BX43, original magnification x20, NA 0.4.

iii) IMPROVED IMAGE FLATNESS (Figures 6 and 7).

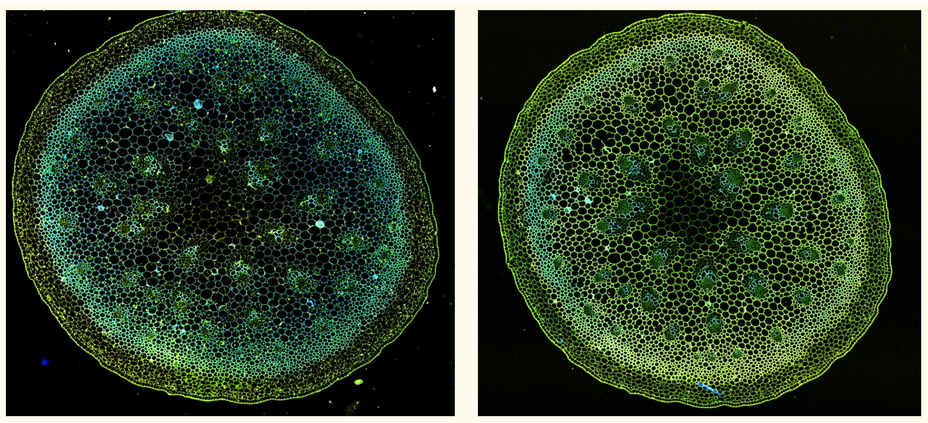


Figure 6: Low power overview images of lily stem tissue prepared on a standard microscope slide (left) and on the CalaClear® microscope slide with black sealant (right). Whole slide fluorescence scanning using InnoQuant Image Acquisition & Image Processing, 0.5µm/pixel (equivalent to NA 0.4)

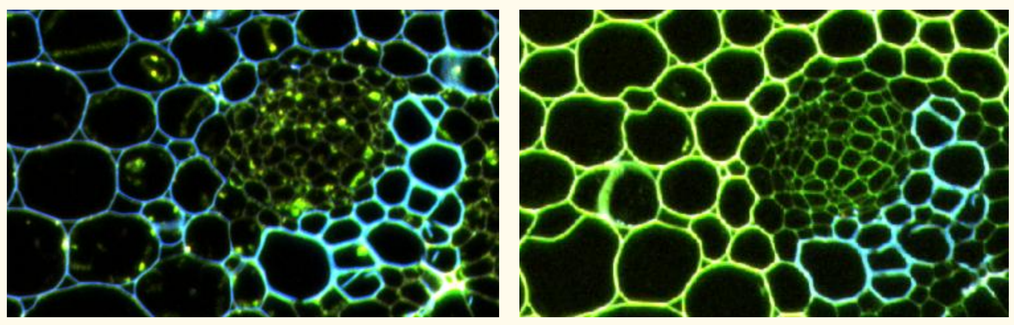


Figure 7: High power detail mages of lily stem tissue prepared on a standard microscope slide (left) and on the CalaClear® microscope slide with black sealant (right). Whole slide fluorescence scanning using InnoQuant Image Acquisition & Image Processing, 0.5µm/pixel (equivalent to NA 0.4)

iv) VIRTUAL THINNING (Figure 8).

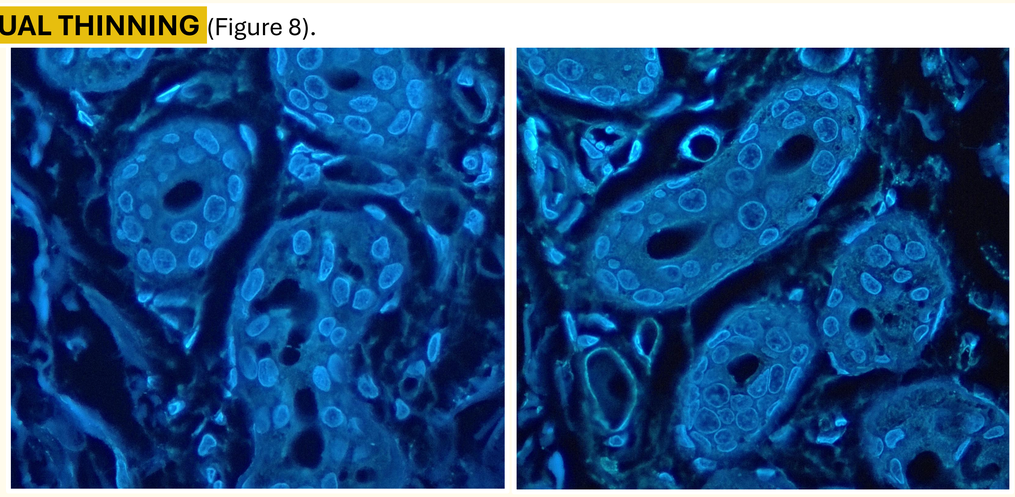


Figure 8: Images of normal sweat glands prepared on a CalaClear® microscope slide with clear sealant (left), and on a CalaClear® microscope slide with black sealant (right) . Olympus BX53, original magnification x40, NA 0.7.

Conclusions

Through a fundamental redesign of the microscope slide, we have achieved superior image quality and standardisation.

Using fluorescence microscopy, we illustrate the enhancement of the four important image quality parameters, viz:
i) Enhanced resolution
ii) Enhanced contrast
iii) Improved image flatness
iv) Virtual section thinning

We are aware of the importance of this advancement to the burgeoning field of digital pathology, particularly in the application of analytical algorithms.

Acknowledgements

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- CPI Ltd for acquiring and evaluating images of pollen on standard and CalaClear® slides.

References

1. Spherical aberration tutorial: https://www.youtube.com/watch?v=06PtwR2IKcw
2. https://www.innopsys.com/product/corporate/innoquant/

Keywords

Fluorescence microscopy, light microscopy, microscope slide, histology, image standardisation, image resolution, image contrast, image flatness.

CalaClear® microscope slide - GB patent granted GB2523774A, EP Unitary patent granted EP3114523, US Patent Application No. 16/549,363