

ILLUMIN8

The newsletter for microscope users

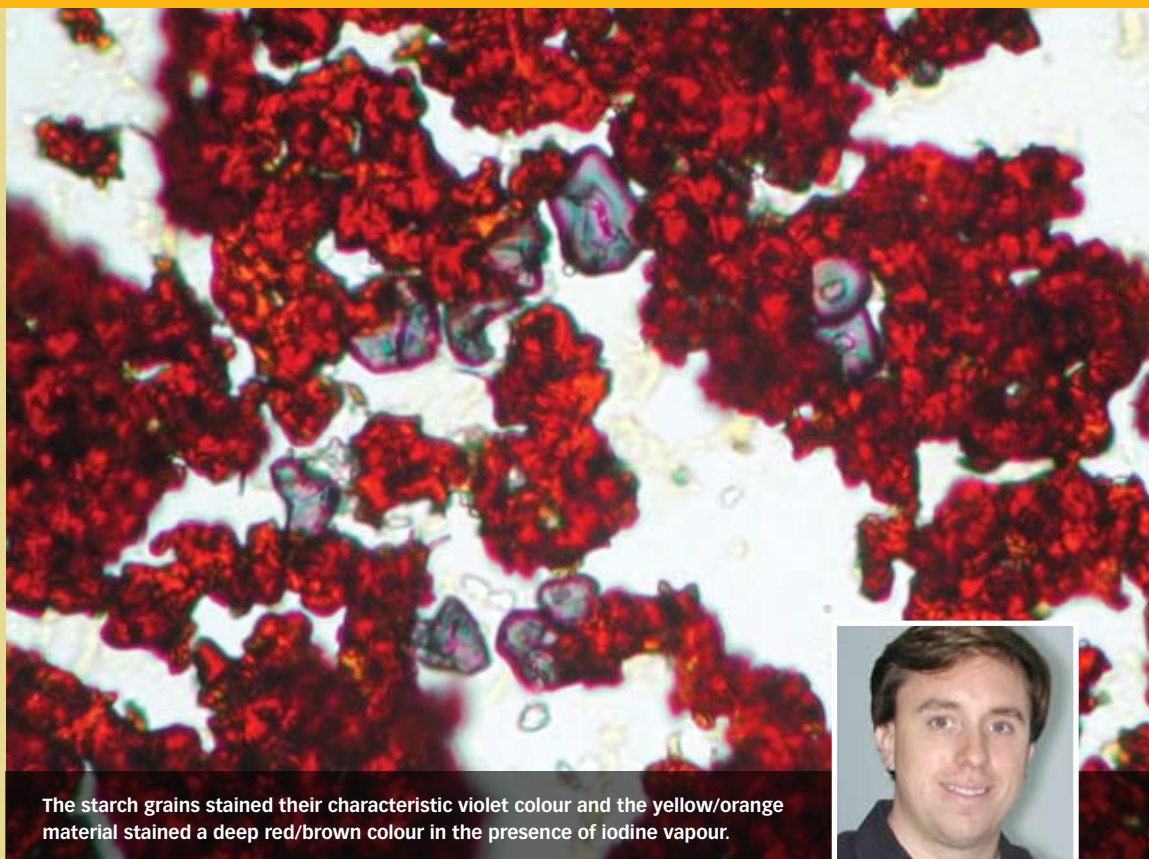
Welcome

This issue of Illumin8 focuses on the use of microscopes in the forensic setting. For future issues we would like to know what you want to read about. Moreover if you are doing any interesting research, or have a great microscopy tip, then send an email to microscopy@olympus.uk.com or fill in the reply paid card. You can also use these to request your own copy of 'Illumin8' as well as the handy leaflet and poster series available. We hope you enjoy this issue and don't miss our competition to win

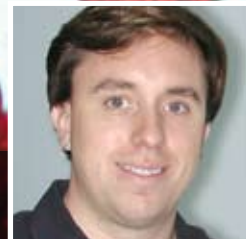


IN THIS ISSUE

- **Forensic Trace Evidence: big picture clues from microscopic particles**
Microscopes in forensic action
- **The microscope enters the field of forensics**
The first forensic microscopy investigation
- **Forensics at the cutting edge**
Pushing forensics forward



The starch grains stained their characteristic violet colour and the yellow/orange material stained a deep red/brown colour in the presence of iodine vapour.



Forensic Trace Evidence: big picture clues from microscopic particles

The Author, Dr Christopher S. Palenik, from Microtrace, a private microchemical laboratory based outside of Chicago that specialises in forensic microanalytical problem solving.

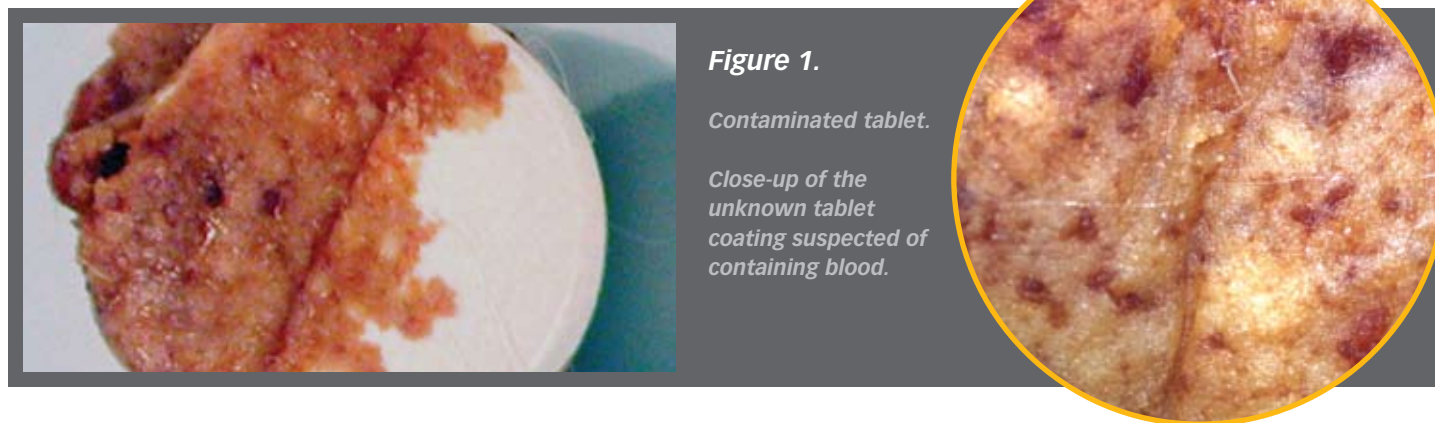
Amidst a forest of instruments growing in complexity, precision, and cost, the polarizing light microscope has remained a fixture of the forensic trace evidence laboratory since the inception of forensic analysis.

The reason for this constancy is not a result of budgetary constraints or nostalgia but rather practical functionality in the realms of both visual observation and the acquisition of analytical data. As poignantly demonstrated in Brian J. Ford's article below, the light microscope has the capability to provide specific answers (not just data) through visualisation both quickly and accurately. This article will build on Brian's historical example by illustrating the way polarizing light microscopy (PLM) can provide information unattainable by other methods and improve the quality of information obtained by complementary analytical methods. The example presented here is whether or not a red residue

on a tablet is, in fact, blood; and if not, what is it? Though the question is straight-forward, the answer is not one that can be answered satisfactorily by any routine instrument-based protocol; yet, this particular question can be answered specifically and eloquently through observations based around light microscopy.

Light Microscopy in Forensic Trace Evidence

In a matter of speaking, light microscopes are pervasive in forensic laboratories; present as low magnification stereo microscopes to locate trace evidence; as comparison microscopes used in hair, fibre and bullet examinations; and coupled to various types of spectrometers to reduce analytical volumes. Within this list, the most overwhelmingly dominant use of the microscope is for either magnification or comparison of morphological features between questioned and known samples. While valuable



enough as a comparator, the light microscope emerges as an even more powerful tool in an old role that is regaining interest; namely, the concept of developing investigative leads. The idea of providing investigators with new facts based on the observation of microscopical evidence is not entirely new; however, redoubled efforts in solving cold cases, hot cases, or in the "war on terror", has opened a new era for the light microscope in which the range of materials that can be examined and the variety of information that can be obtained is being rediscovered.

Investigative Microscopy

In our laboratory, the light microscope is always the first tool used to examine an unknown sample. In particular, when no sample is available for comparison, or a factual identification must be established, the light microscope becomes even more important. In the case to be related here, a sticky, orange/red residue was found on the surface of the pharmaceutical tablet shown in Figure 1.

Presumptive tests for blood showed that none was present. At this point, the most significant questions to be asked were: what is this residue and when was it introduced?

An FTIR spectrum of the neat residue suggested only a mixture dominated by a carbohydrate spectrum, which provided little information towards the final answer. Microscopical observation, however, through a simple, temporary preparation of a portion of this sample in immersion oil, indicated that this residue consisted of at least three components (Figure 2).

The most recognizable component was starch, which can be readily identified on the basis of the "maltese cross" seen when observed between crossed polars. Based on its morphology, the starch can be more specifically identified as corn starch.

Interestingly, when staining the starch with iodine vapour for secondary confirmation, it was noted that the second component, an isotropic orange substance stained a deep orange colour (Figure 3 on front cover). This was the first

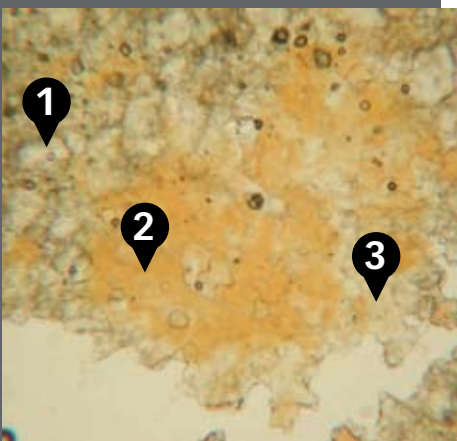
clue that suggested the composition of this material was povidone, which is known to stain a deep orange/brown colour in the presence of iodine. This hypothesis was confirmed when a portion of the pure orange material was microscopically isolated and identified by FTIR microspectroscopy.

The final component, a colourless, euhedral, birefringent substance was well mixed throughout the residue. The material was isolated through micro-extraction and recrystallization from water. The resulting phase, which was only noticed by light microscopy, could then be identified conclusively by Raman microspectroscopy as Vitamin C, ascorbic acid.

Ultimately, it was determined that the sticky residue resulted entirely from manufacturing ingredients and deposited after the tablet was pressed. More specifically, povidone, which is extremely hygroscopic, came into contact with water or water vapour and caused the tacky texture of the residue allowing it to adhere to the previously pristine tablets. Finally, the orange colour of the residue resulted from a partial degradation of the povidone, most likely due to exposure to increased temperature. Laboratory experiments confirmed the yellowing of povidone in the presence of heat. Together, these conclusions not only answered the specific question, but provided investigative information leading to understanding the source of the problem.

Figure 2a

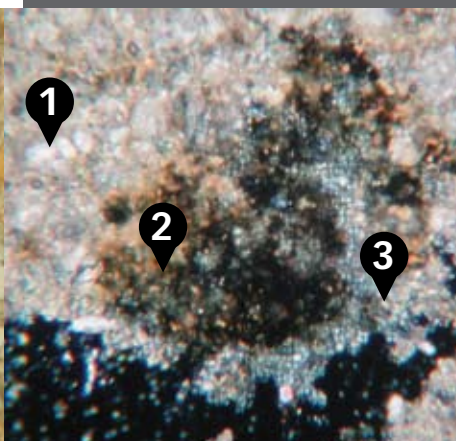
Image of a small area of the orange/brown coating. The majority of the coating is a colourless to slightly yellow material that consists of starch and ascorbic acid.



- 1 - Ascorbic Acid
- 2 - Orange Isotropic Component
- 3 - Starch Grains

Figure 2b

Same field of view as Figure 2a but as observed between crossed polars. The "Maltese" cross of the starch grains, isotropic orange compound and euhedral birefringent crystals can all be observed.



- 1 - Ascorbic Acid
- 2 - Orange Isotropic Component
- 3 - Starch Grains

Chance Favours the Prepared Mind

To most present day forensic laboratories, a case (or analysis) such as this might be considered unusual, or more likely overkill. The context of this forensic case is unusual by criminal forensic standards, as are the materials encountered, and the extent of questions asked of this evidence. However, the value of investigative information (in this case regarding the nature and source of the contamination) is becoming of increasing interest to investigators in a variety of forensic disciplines. For the forensic scientist, this example illustrates that while instrumentation plays an undeniably important role in the modern laboratory, it is only through careful observation that we can know what to analyse and how to interpret these results. To this end, the light microscope provides invaluable assistance.

The microscope enters the field of forensics

Analytical forensic microscopy has a contemporary ring, and the example recounted by Chris Palenik in this issue shows how useful the optical microscope can be. Yet this approach reaches back to the roots of microscopy. Leeuwenhoek first turned his microscope to forensic ends 320 years ago, and used it with an honesty that we can admire today.

In his letter to the Royal Society of 17 October 1687 he explained that he had been sent a specimen by a correspondent in the Baltic state of Courland. It was a sample of 'heavenly paper', a blackened piece of papery material that was believed to be a letter from the heavens (and seemed to have been charred by what we would call the heat of re-entry). The specimen was one of those I examined after finding it in its original envelope in the Royal Society's archives.

Leeuwenhoek says that he examined the specimen under his home-made microscope and soon determined that it wasn't paper, but was a dried film of what we'd now know as chlorophyte algae. He described it as green plant material that came forth from water.

Although his diagnosis was right, Leeuwenhoek felt that he needed the substantiation of experiment. He took some of the green algal material that he found growing in a canal in his native Delft and dried it for himself. Then he took another specimen, this time of algae from a rain-barrel and dried it in front of the fire. Under his microscope he could tell that the three specimens had similar appearance. Thus, not only did he make the right identification, but he confirmed it using experimental microscopy.

I took fragments of these original specimens and reconstituted them in sterilized water to reveal the algae that Leeuwenhoek had studied. Most of the original 'heavenly paper' was made up of filaments of Urospora. In the

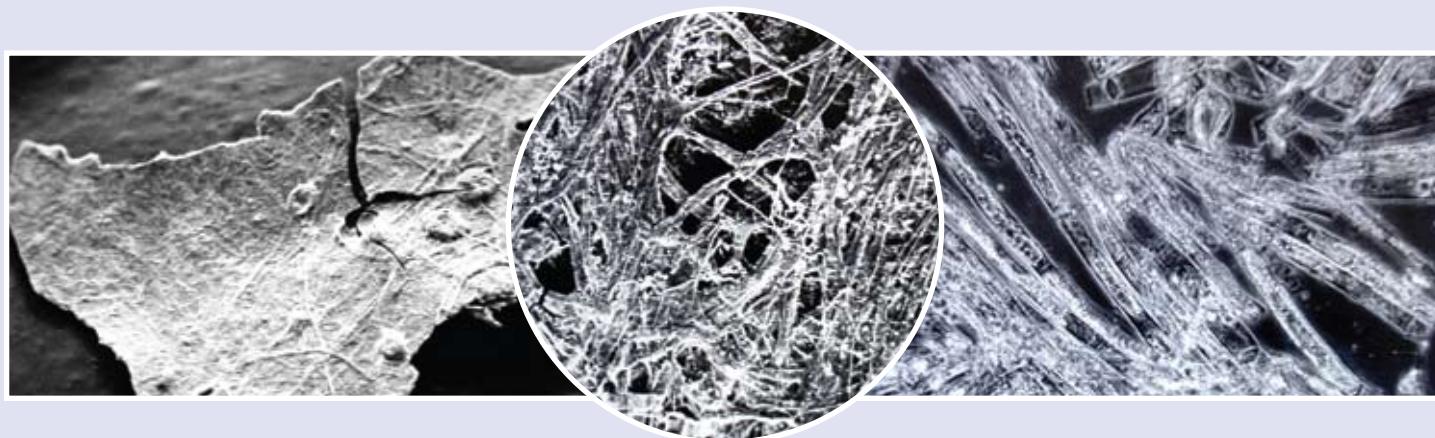
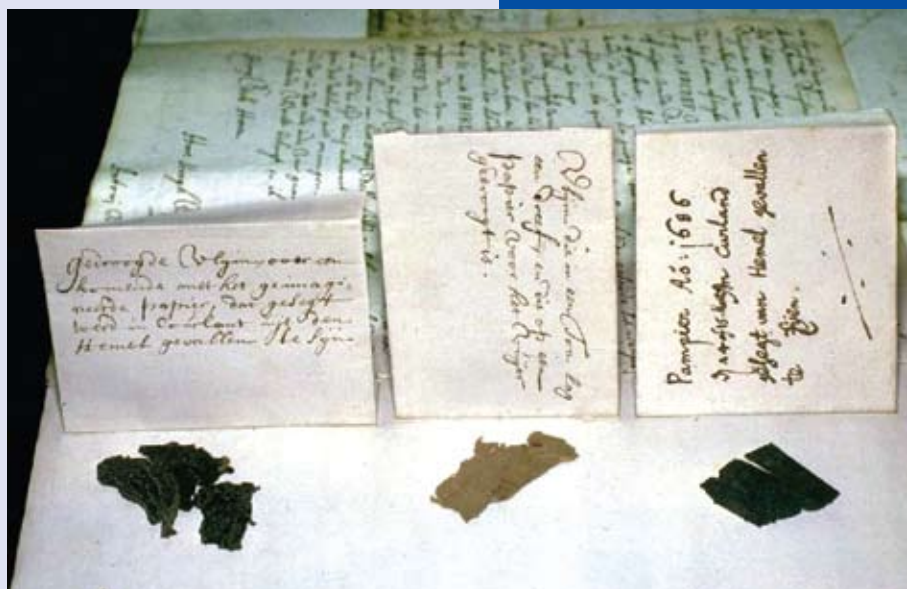
others I found Rhizoclonium and Oedogonium. Later I examined fragments of the original dried specimens under the scanning electron microscope at Cardiff University. I could see the strands of algae clearly. One of the technicians, who had worked on paper for many years, came in and said: 'Ah, paper specimens! I'd know that sight anywhere.' But she was wrong. The filamentous algae clearly showed the transverse cell walls that are absent from paper fibres.

It is testimony to Leeuwenhoek's abilities that, using a single-lens microscope in the seventeenth century, he could identify a specimen that was mistaken even by a present-day technician with the benefits of scanning electron microscopy. His objectivity established a long line of investigative microscopists, and we can witness Leeuwenhoek's legacy today



Brian J Ford, photographed here lecturing at Inter Micro 2007 in Chicago, is based at Gonville & Caius College, University of Cambridge. He has been a Fellow of the Royal Microscopical Society since 1962. Among his books is *Leeuwenhoek Legacy* (1991). Image courtesy of Hazel Bales, Microtrace

Three packets of papery specimens were sent by Leeuwenhoek to London on 17 October 1687. The one on the right was 'heavenly paper', the others are the versions he prepared to test his hypothesis about how the 'paper' had been produced.



This fragment of Leeuwenhoek's home-made 'heavenly paper', when examined under today's scanning electron microscope, shows algal filaments clearly. The rounded bodies that are also visible are water-fleas trapped among the chlorophyte algae. Field width 4 mm

At first appearance the specimens could be identified as paper, but transverse septa in each filament betray the real nature of the material - elongated chlorophyte algal cells joined end to end. When growths like this are stranded as a pool dried up, a papery substance forms. Field width 1 mm.

Under phase contrast using an Olympus microscope, this reconstituted specimen shows algal cells clearly. Each cell contains a ribbon-like chloroplast indicative of the freshwater genus *Mougeotia*. This genus is common in Dutch canals (*Illumin8* No 10). Field width 500 μ m

Forensics at the **cutting edge**

Cut to the chase

Genetic fingerprinting was developed by Professor Alec Jeffries at the University of Leicester in 1984 and has become an essential tool in many areas, but none more so than the fight against crime. Recent advances have made it possible to apply the technique to even a very small number of cells. In some situations though, obtaining a clean sample from the plethora of suspect and victim cells has proved a barrier to forensic detection. Laser microdissection (LMD) is now being used to overcome this barrier, providing a simple, safe and contamination free method of extracting the target cells for downstream analysis. Furthermore, new fluorescent cell identification processes can be used ensuring that male and female cells are easily distinguished even if they are not reproductive cells. This, coupled with extraction using LMD instruments such as the Olympus CellCut Plus and SmartCut Plus, produces a formidable forensic tool.

Advanced amplification

Advalytix (an Olympus company) is introducing a superior amplification platform for ultra-low volume (1 μ L range) or single cell applications, based on a chemically structured microscope slide. With this innovative system, sensitivity, efficiency and reproducibility are reliable constants for single cell analysis, low-copy number approaches and ultra-low volume amplification techniques. And as a result fast and convenient workflow is assured. The AmpliGrid system is the key to obtaining perfect results especially with single cells and nearly any other kind of template material e.g., forensic stains or purified nucleic acids. This system is setting a new

standard for low volume and single cell assays. Identity testing using human DNA is a growing field in molecular biology and especially forensics. Several commercial kits and products based on the analysis of short tandem repeats (STR) of nucleic acids are available. Using the AmpliGrid technology it is now possible, for the first time, to apply these kits confidently to even single cell reactions. Systematic studies to improve sensitivity, characterise drop-outs, analyse cells in a population one by one, as well as many more approaches, have shown to be excellent new methods in the precise typing of single cells.

The devil is in the detail

The new Olympus LEXT OLS3100 metrology microscope (confocal laser scanning microscope) has been a great hit with material science researchers and can now boast its place in the forensic toolkit. It is presently being used for bullet investigations in an international trial, since its high resolution and magnification capabilities are coupled with its ability to image objects without any preparation and using advanced 3D analysis. This enables extremely accurate measurement of surface features, including depths, lengths, angles and volumes as well as roughness and many other features. This means that even the faintest of identifying marks can be easily analysed and more importantly compared.

To find out more about any of the Olympus products or services mentioned, please email microscopy@olympus.uk.com



Olympus CellCut Plus



Olympus Advalytix AmpliSpeed



Olympus LEXT OLS3100



Competition time: Your chance to win an Olympus μ[mju:] Digital Camera

The stunning 12 Megapixel Olympus μ[mju:] 1200 is waterproof and produces ultra-sharp prints in poster-size and beyond. Innovations such as Face Detection Technology ensure your pictures impress with their realism; while the 6.9cm HyperCrystal LCD makes framing and reviewing shots with friends more enjoyable. We are giving one away, so to be in with a chance of winning you will need to answer the three questions below correctly on the reply paid card and return it to us by the 3rd December 2007.

Question 1:

What type of microscopy is most used by Chris Palenik?

Question 2:

What did Leeuwenhoek discover the 'Heavenly paper' to really be?

Question 3:

Who invented genetic fingerprinting?

Congratulations to Mr A Bendall, from the Dept of Microbiology at Ipswich Hospital, for winning the Olympus WS-331M Voice Recorder from the last issue of Illumin8.



OLYMPUS

Your Vision, Our Future

To request further information:

Olympus UK Ltd
Vision House
19 Colonial Way
Watford
Herts
WD24 4JL

Tel: 01923 831100

Fax: 01923 201767

Email: microscopy@olympus.uk.com

Web: www.olympus.co.uk

Title & Name _____
Dept. _____
Institute/Company _____
Address _____

Telephone _____
Email _____

My answers to the competition are:

- (1) _____
(2) _____
(3) _____

Please send me future issues of Illumin8 by post by email

Please send me the leaflets on: Darkfield

Focus lock Koehler Illumination

Phase Contrast Cleaning & Maintenance

Please contact me about

Olympus forensic science solutions

Please send me more information on:

Suggestions for future issues of Illumin8:

I would like to receive regular Olympus eNewsletters

Nov 07

Title & Name _____
Dept. _____
Institute/Company _____
Address _____

Telephone _____
Email _____

My answers to the competition are:

- (1) _____
(2) _____
(3) _____

Please send me future issues of Illumin8 by post by email

Please send me the leaflets on: Darkfield

Focus lock Koehler Illumination

Phase Contrast Cleaning & Maintenance

Please contact me about

Olympus forensic science solutions

Please send me more information on:

Suggestions for future issues of Illumin8:

I would like to receive regular Olympus eNewsletters

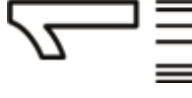
Nov 07

Business Reply Plus
Licence Number
RRLT-UUBS-RLUE



OLYMPUS UK LTD
VISION HOUSE
19 COLONIAL WAY
WATFORD
WD24 4JL

Business Reply Plus
Licence Number
RRLT-UUBS-RLUE



OLYMPUS UK LTD
VISION HOUSE
19 COLONIAL WAY
WATFORD
WD24 4JL