

# Fundamental Knowledge

## Part 3 From Theory to Reality

In parts one and two (I&M Volume 9 issue 4 & volume 10 issue 1) of this chapter we looked at the physical principals of light and its interaction with optical surfaces. We then went onto consider how these underlying principles manifest in the capabilities and limitations of microscope optical systems. In this part of the chapter, we look at how these principles are put into practice – essentially we address the reality of microscopy for industrial applications.

Successful imaging and analysis must always start with the correct sample preparation and understanding your microscope. There are myriad reference texts on sample preparation for all possible sample types so we will not attempt to address this diverse topic here. Understanding your microscope and the imaging methods available though, is certainly within the scope of this series of articles.

### Types of Microscope

Generally speaking, light microscopes can be divided into two main types of optical system: compound and stereo. But in effect, compound microscopes are composed of either upright or inverted systems and there are two types of stereo system. There are divisions beyond this

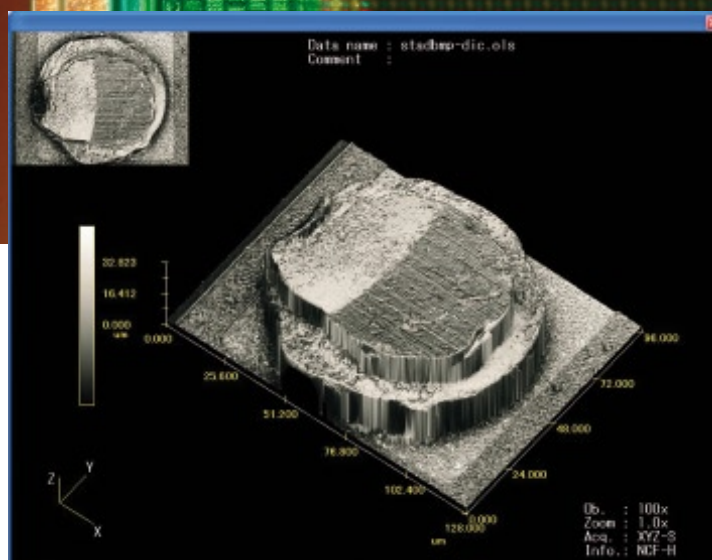


Fig. 1: Laser DIC (in 3D) of a stud bump.

mostly based on use, e.g routine or research. Routine microscopes tend to be less flexible and therefore smaller in size, whereas research microscopes can be adapted and added to, making them larger.

#### Upright

Upright microscopes have the objective lens positioned above the sample stage, so the light path can be very simple, travelling straight from the sample to the imaging system (eyes or camera). Modern microscopes are slightly more complex, partly for versatility and partly for ergonomomy purposes.

#### Inverted

Inverted microscopes have the objective lens positioned below the sample stage, and so the light path is often more convo-

luted then with an upright microscope. This does though, generally offer greater flexibility for placing components into the light path, such as illumination and camera options.

#### Stereo

Stereo microscopes can be sub-divided into two distinct optical systems. The Greenough system consists of two inwardly inclined pathways converging at the object stage, with separate optical components perpendicular to the light paths (see fig. 6). This type of system provides very compact microscopes, excellent for investigation of samples with plenty of depth information, such as gears and manufactured components. For samples with less depth information e.g. materials surface analysis, a Greenough system is not ideal.

Fig. 2: Reflected light DIC of a CCD chip.

The Galilean system, on the other hand, is much more complex. As soon as the light paths enter the front of the single common objective lens, they are refracted to travel in parallel through the rest of the objective lens, the zoom body and into the observation tubes. The parallel nature of the light enables the addition of intermediate tubes, such as coaxial reflected light and fluorescence illuminators, beam splitters, eye point adjusters, discussion tubes and aperture diaphragms, amongst others. In the observation tube, a series of prisms change the separation distance and convergence angle of the light paths to put the images through the eyepieces. This type of stereo has a larger form factor than a Greenough system, but is very flexible and provides excellent results across all applications.

### Generating Contrast

Microscopes of all types are designed to collect light from a sample and magnify the image using a series of specially designed lenses, projecting the focused image for either the human eye or a camera to view. To achieve this, the sample or illumination method must produce contrast so that the imaging system is able discern differences within the sample. There are many different methods used to generate contrast in microscopy and we shall only scrape the surface of the most relevant methods for industrial applications. Therefore, we suggest that for further information you visit the excellent <http://www.microscopy.fsu.edu/primer/> website.

### Brightfield

The most commonly used method throughout all forms of light microscopy

(from life sciences through medicine to materials analysis and industrial uses) is bright field. This is where white light is either transmitted through a sample or reflected off a surface (see fig. 4 for a schematic of a standard reflected illumination pathway) and the properties of the sample itself produce the contrast required. This is essentially a scaled up version of how our own imaging systems work – the differential absorbance of various wavelengths of light by the sample produces distinct colour contrasts. So it is easy to distinguish between bits of the sample that absorb different wavelengths of light. In many cases this magnified view of the sample is enough for the imaging and analysis systems to glean the appropriate information. Bright field is also universally accessible for upright, inverted and stereo microscope systems.

### Darkfield

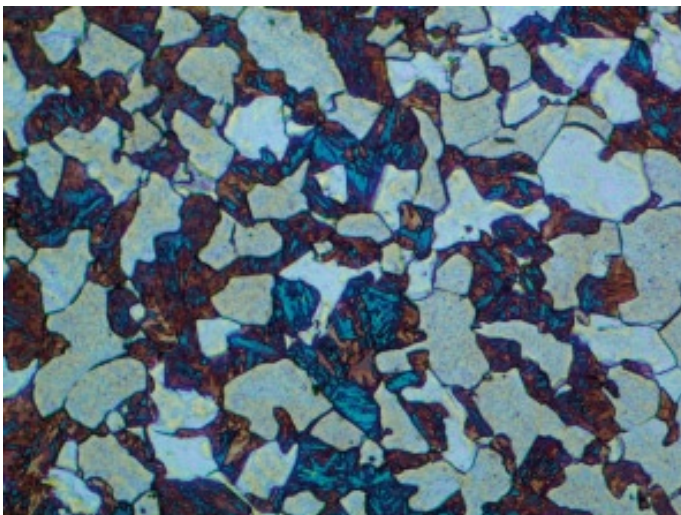
Where bright field contrast is essentially a subtractive process, dark field is a disruptive one. The sample is illuminated at oblique angles, such that no reflected or transmitted light is collected by the objective. Only light diffracted or 'scattered' by features in the sample or on the surface can enter the objective. This means that the majority of the field of view will be black since no light information is being generated by it. Elements of interest, where light is suitably diffracted will show up as bright points. This type of microscopy is excellent for identifying scratches on otherwise smooth surfaces, for instance. It is also very useful for identifying grain boundaries in heterogeneous alloys. Importantly, dark field imaging can be generated on conventional (upright and inverted) as well as stereo

systems enabling users to gather information on feature height data as well as occurrence (see fig. 5 schematic of dark-field illumination).

### Polarizing Light Microscopy

Polarized light is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials (those that produce two beams of polarised light in perpendicular planes) when compared to other techniques such as dark field, bright field illumination and differential interference contrast, for example. Polarized light microscopes have a high degree of sensitivity and can be utilized for both quantitative and qualitative studies targeted at a wide range of anisotropic specimens. Qualitative polarizing microscopy is very popular in practice, with numerous volumes dedicated to the subject. In contrast, the quantitative aspects of polarized light microscopy, which is primarily employed in crystallography, represent a far more difficult subject that is usually restricted to geologists, mineralogists, and chemists. However, steady advances made over the past few years have enabled biologists to study the birefringent character of many anisotropic sub-cellular assemblies.

Optically, polarizing light microscopy needs a pair of polarizing elements (a polarizer and an analyzer). Light passes through the polarizer, producing a beam of linearly polarized light in the same plane (often known as "plane polarized"). The light is focused by the objective lens for reflected polarizing light microscopy (or the condenser for transmitted polarizing light microscopy) and then interacts with elements on the surface of the sample. If the light returns un-affected by the



◀ Fig. 3: Reflected polarised light showing clear grain boundaries.

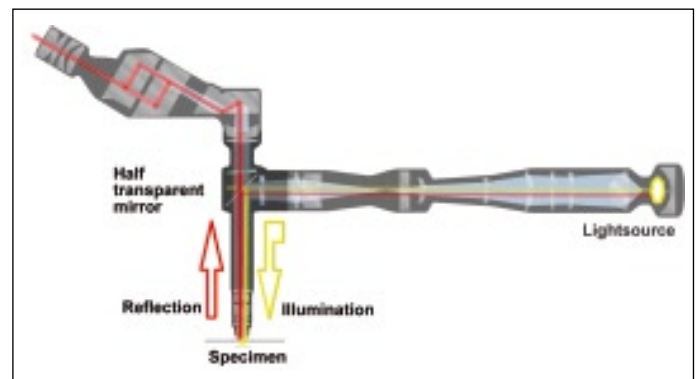


Fig. 4: Schematic of a standard reflected illumination pathway.

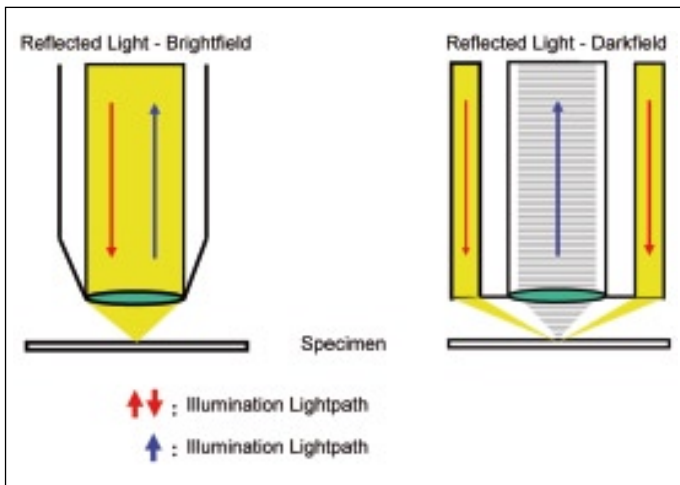


Fig. 5: Schematics of how reflected brightfield and reflected darkfield illumination are handled by the objectives.

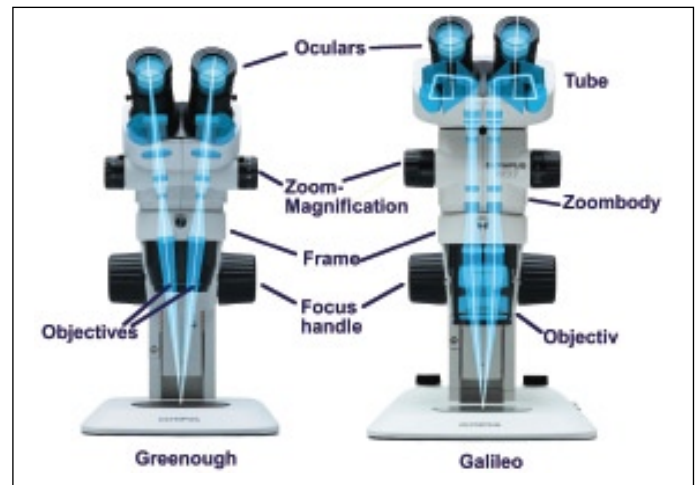


Fig. 6: Overview of Greenough (left) and Galileo (right) stereo microscope light paths.

sample it is blocked by the second polarizer (analyser), as this is placed at right angles to the first polarizer. Elements in the sample that do change the plane of polarization or are birefringent can produce light rays capable of passing through the analyser and therefore create contrast with the surrounding area. Since the illumination is very specific and some light is excluded by the analyser, the background of the view is black and features stand out clearly. Furthermore, elements within samples are displayed in a rainbow of colours depending on the polarization changes they introduce (see fig. 3).

#### Differential Interference Contrast

Differential interference contrast (DIC) microscopy was first described by Nomarski in 1952. The optical properties of DIC are quite complex (compared to brightfield or darkfield for example) and involves the use of a pair of polarizing elements (a polarizer and an analyzer) as per polarizing light microscopy, and two crystalline beam-splitting devices (commonly known as Wollaston prisms) which enable the detection of gradients in optical path length within specimens by converting them into light intensity differences. To achieve this, a light beam passes through a polarizer, producing a beam of linearly polarized light in the same plane, just as in polarized light microscopy. When the polarized light beam enters a Wollaston prism, each incident ray is split into two spatially separated parallel rays, named ordinary and extraordinary rays, vibrating at  $90^\circ$  to each other. The beams are then focused by the objective and impinge on the surface of

the sample. If adjacent regions of the sample differ either in refractive index or thickness then the two beams of rays are delayed or refracted differently resulting in slight optical path differences as well as phase shifts. On passing back through the Wollaston prism, the two rays are recombined into an elliptically polarized light ray and then pass through the second polarizer (analyser), and subsequently generate an amplitude image of the specimen through interference at the image plane. Therefore, the difference in optical path is translated into a change in amplitude visualised as intensity in the final image. Care must be taken in reviewing DIC images though, as they produce a 3D-like effect, but the apparent peaks and troughs seen in the image are not actual representations of the morphology of the surface. Instead, they are the product of the optical gradient through the specimen and the wavefront path distance. So, while DIC images are impressive, their interpretation must be done with knowledge of what the peaks and troughs are actually showing.

DIC is an excellent technique for metallurgy, materials and semiconductor industries, producing good images of surface features such as scratches and components (see fig. 2).

#### Confocal Laser DIC

DIC by its very nature provides a reasonably tight depth of focus and high resolution and so there is scope for imaging different Z sections of a sample to produce an excellent overview of the features. The use of DIC on a confocal laser scanning microscope though, enables even finer Z resolution, as well as increasing

horizontal resolution, to provide very fine surface detail. This is clearly evident on microscopes like the Olympus LEXT OLS3100, which can provide metrology images not normally possible on optical microscopes (see fig. 1).

#### Conclusion

Microscopy is nothing without contrast! As a result, in order to 'image' and analyse images effectively, information from the sample must be visualised using a combination of the sample-preparation, correct microscope, illumination and imaging technique. There are many different ways of using these essential components to generate contrast, but here we have touched only on those methods most relevant to industrial applications: from brightfield and dark field, to polarization and DIC. There is no doubt though, that getting these components right will make a big difference to your imaging capabilities.

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