

Leica DVM - 3D Visualisation

Vertical resolution in the balance between numerical aperture and depth of field

Living up to Life

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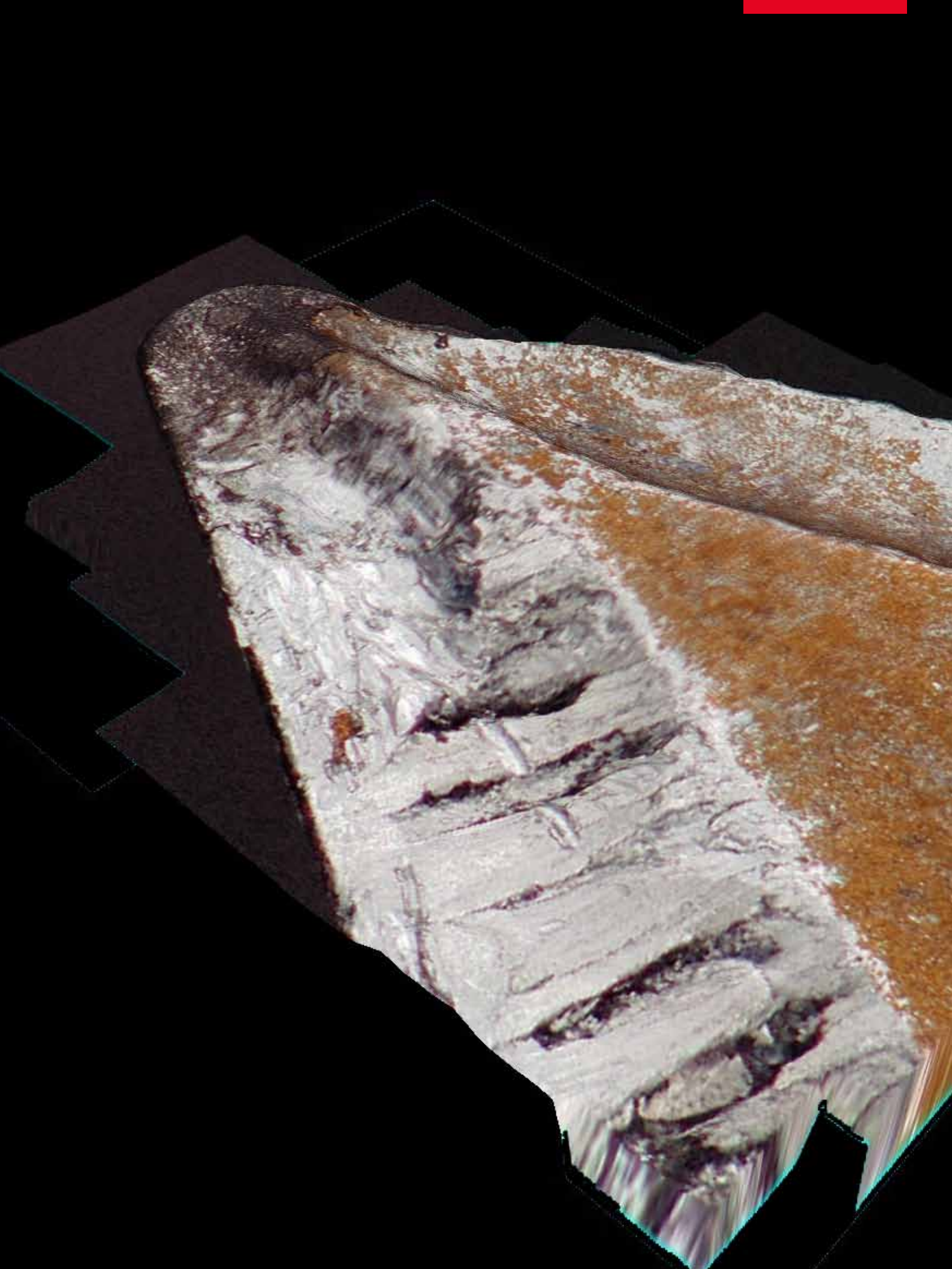
Digital microscopy offers clear advantages for a wide variety of industrial quality inspections, particularly surface analyses. Fracture analyses, analyses of inclined or vertical surfaces or onsite inspections of large parts such as turbine rotors are just a few examples in which the strengths of digital microscopes make the biggest difference. But what are the key criteria for successful use of digital microscopes and which parameters affect the three-dimensional imaging to be expected for these specimens?

One of the main features of a digital microscope is the speed and ease with which it enables surface models to be created of macroscopic and microscopic structures. In a qualitative evaluation, these provide better understanding and documentation of the specimen. In addition, quantification of the surface provides valuable information about the composition of the surface and its wear. Which specimens are suitable for use with a Leica digital microscope, and what are the limitations of the method used?

The three-dimensional imaging of the Leica DVM series is based on the principle of focus variation. The limited depth of field of the optics is utilized to determine depth information for the specimen. Vertical movement of the specimen relative to the objective determines the focus information along with the distance to the optics. For each vertical position, the area of the image that is in sharp focus is separated from the blurry area, and both are processed by the software to create a surface model. One of the advantages of this method is that in addition to the height information, the texture of the specimen is also documented.

Which influencing factors are determinative for successful creation of a 3-D surface model and how do these variables influence lateral and vertical resolution?





Optics and Surfaces

Depth of field

The author of the first publication on the subject of visually perceived depth of field was Max Berek, who published the results of his extensive experiments as early as 1927. Berek's formula gives practical values for visual depth of field and is therefore still used today. In simplified form, it is as follows:

$$T_{\text{VIS}} = n \cdot \left[\frac{\lambda}{(2 \cdot NA^2)} + 340 \mu\text{m} / (NA \cdot M_{\text{TOT VIS}}) \right]$$

T_{VIS} : Visually perceived depth of field

n : Refractive index of the medium in which the specimen is situated. If the specimen is moved, the refractive index of the medium that forms the changing working distance is entered in the equation.

λ : Wavelength of the light used; for white light, $\lambda = 0.55 \mu\text{m}$

NA : Specimen-side numerical aperture

$M_{\text{TOT VIS}}$: Visual total magnification of the microscope

If in the equation above, we replace the visual total magnification with the relationship of the useful magnification ($M_{\text{TOT VIS}} = 500 \text{ bis } 1000 \cdot NA$), it becomes clear that in a first approximation, the depth of field is inversely proportional to the square of the numerical aperture.

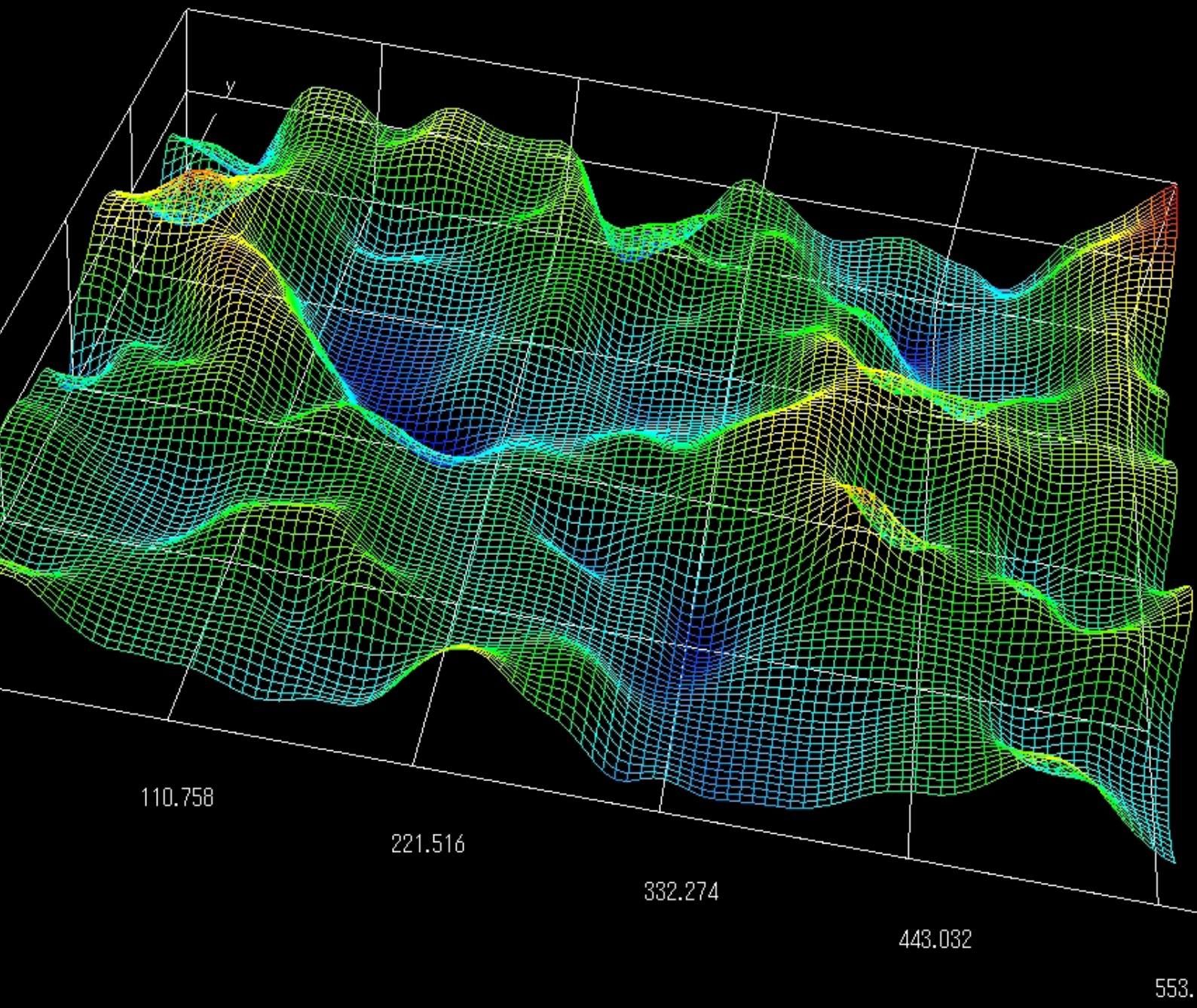
1. Optics – in the balance between numerical aperture and depth of field

In microscopy, depth of field is in many cases an empirically understood metric. In practice, the correlations between the numerical aperture, resolution and magnification determine this parameter. With their adjustment options, today's microscopes create a balance between depth of field and resolution that is optimal for the visual impression – two parameters that in theory are inversely correlated. In DIN/ISO standards, the specimen-side depth of field is defined as the "axial depth of the space on both sides of the specimen plane in which the specimen can be moved without detectable loss of sharpness in the image focus, while the positions of the image plane and objective are maintained."

However, the standard does not give any clues on how to measure the detection threshold of the deterioration of focus. Particularly at low magnifications, the depth of field can be significantly increased by stopping down, i.e. reducing the numerical aperture. This is usually done using the aperture diaphragm or a diaphragm that is on a conjugated plane to the aperture diaphragm. However, the smaller the numerical aperture, the lower the lateral resolution. Thus it is a matter of finding the optimum balance of resolution and depth of field depending on the structure of the specimen.

2. Texture of the specimen

The texture of the specimen surface, also known as its composition, encompasses all of its features and characteristics. These include color and brightness characteristics of the surface. As described above, the principle of focus variation is based on the methodical approach of a lack of specimen sharpness. The better the specimen can be divided into sharp and out-of-focus areas, the better the results of the surface model will be. This method is particularly well suited to textures that have a good contrast. As in many application areas of microscopy, the illumination is given an especially important status, as it frequently determines success or failure. Selecting a suitable illumination makes it possible to document even a specimen with little texture. For example, you can select an oblique incident illumination that makes even hidden structures visible.



Mechanics and Illumination

3. Mechanical resolution in the vertical direction

The third influencing factor in this equation is the mechanical resolution in the vertical direction. This term means the smallest possible steps in the z-direction of the focusing drive, which is usually motorized. To make full use of the performance capacity of the optics, the smallest possible step must be smaller than the currently used depth of field, as otherwise image data are lost. A motorized focus drive with a resolution of 10µm, for example, is suitable at a depth of field of 15µm.

Which lateral and vertical resolutions are possible with a Leica DVM system? As described above, these parameters depend on various influencing factors, such as the surface structure or illuminator, and thus must be determined depending on the application. Interpolation attains a vertical resolution of one-half of the applied depth of field. The lateral resolution is determined by the numerical aperture of the magnification used.

Illumination

Selecting the suitable illumination is critical to the success of the examination. The modular structure of the Leica DVM product concept enables you to combine the selected optics with the optimal illumination for the application. The following methods are available for selection:

- 1) Variable oblique incident illumination: This method changes the illumination direction from vertical to lateral. This approach is particularly suitable for visualizing scratches or small recesses.
- 2) Diffuser: For shiny surfaces, the dynamic range of the camera is insufficient in many cases and many areas of the specimen are overexposed. A diffuser provides reliable reduction of the overexposed area.
- 3) Coaxial illuminator: A coaxial illuminator is used for very shiny or reflective surfaces, such as wafers or metal sections.
- 4) Polarized light: is used to suppress the reflections or for documentation of plastic materials.
- 5) Coaxial illuminator with directed light: In the applications described above, the directed light creates a three-dimensional impression of the specimen. This is helpful in many cases for determining the surface with greater accuracy.



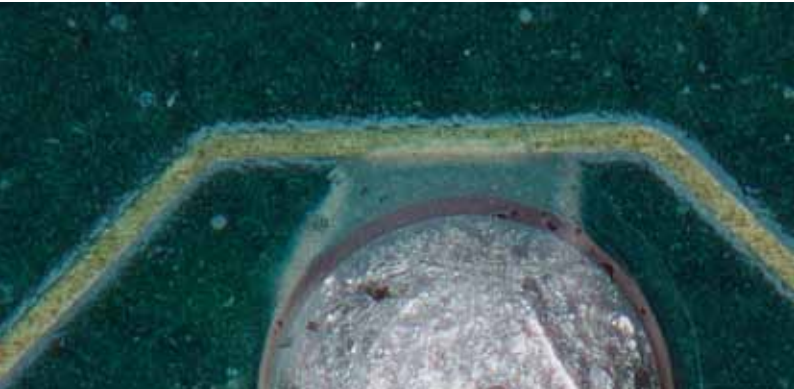
Coin with incident illumination



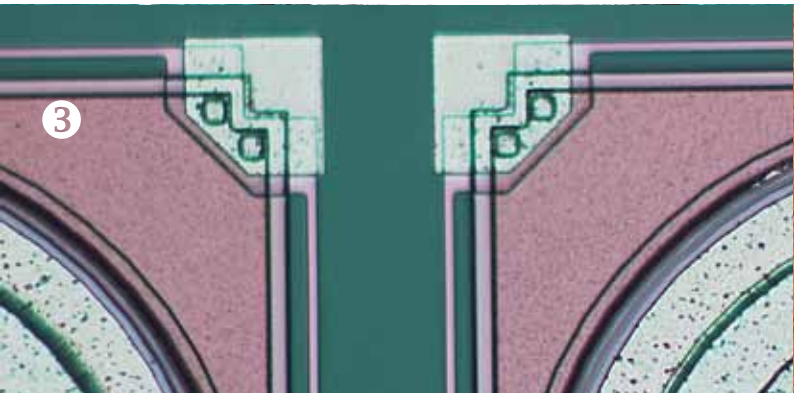
Coin with **oblique incident illumination**



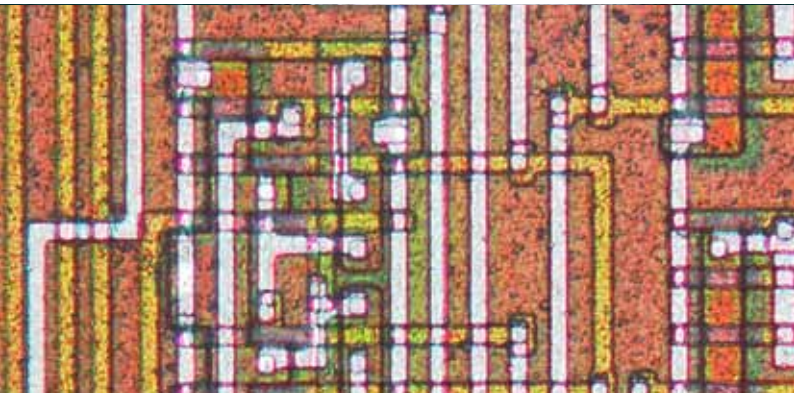
Soder joint without diffuser



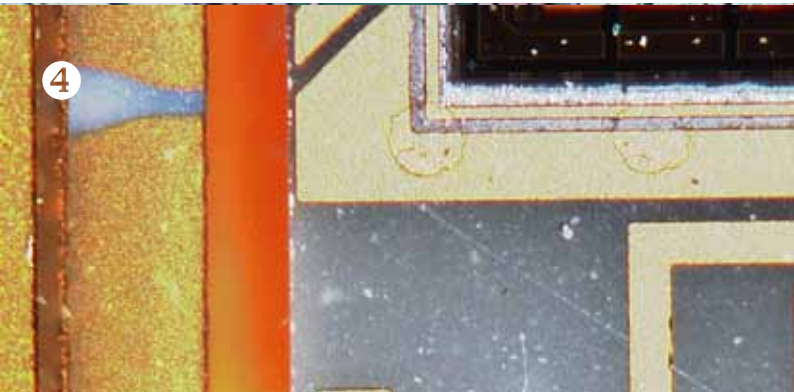
Soder joint with **diffuser**



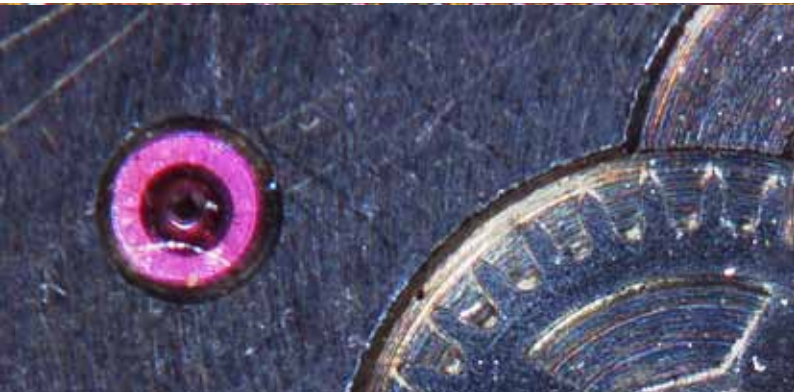
Semiconductor with **coaxial illumination**



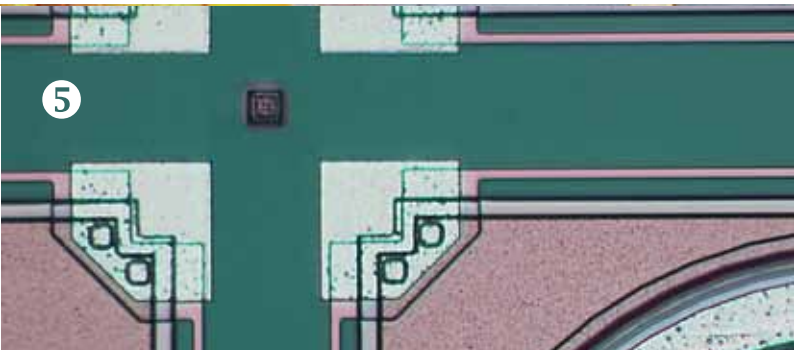
Semiconductor with **coaxial illumination**



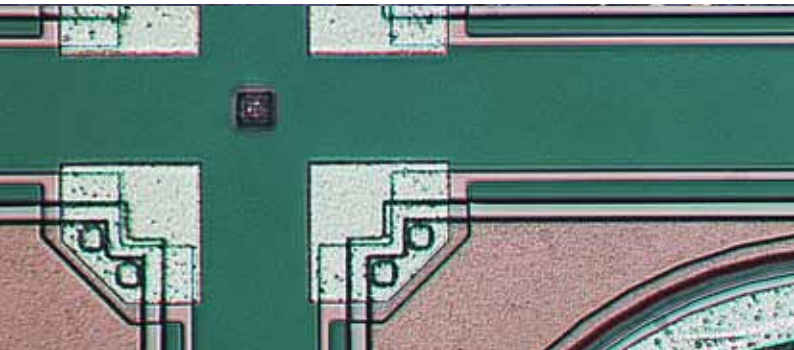
Plastic with **polarised light**



Watch with **polarised light**



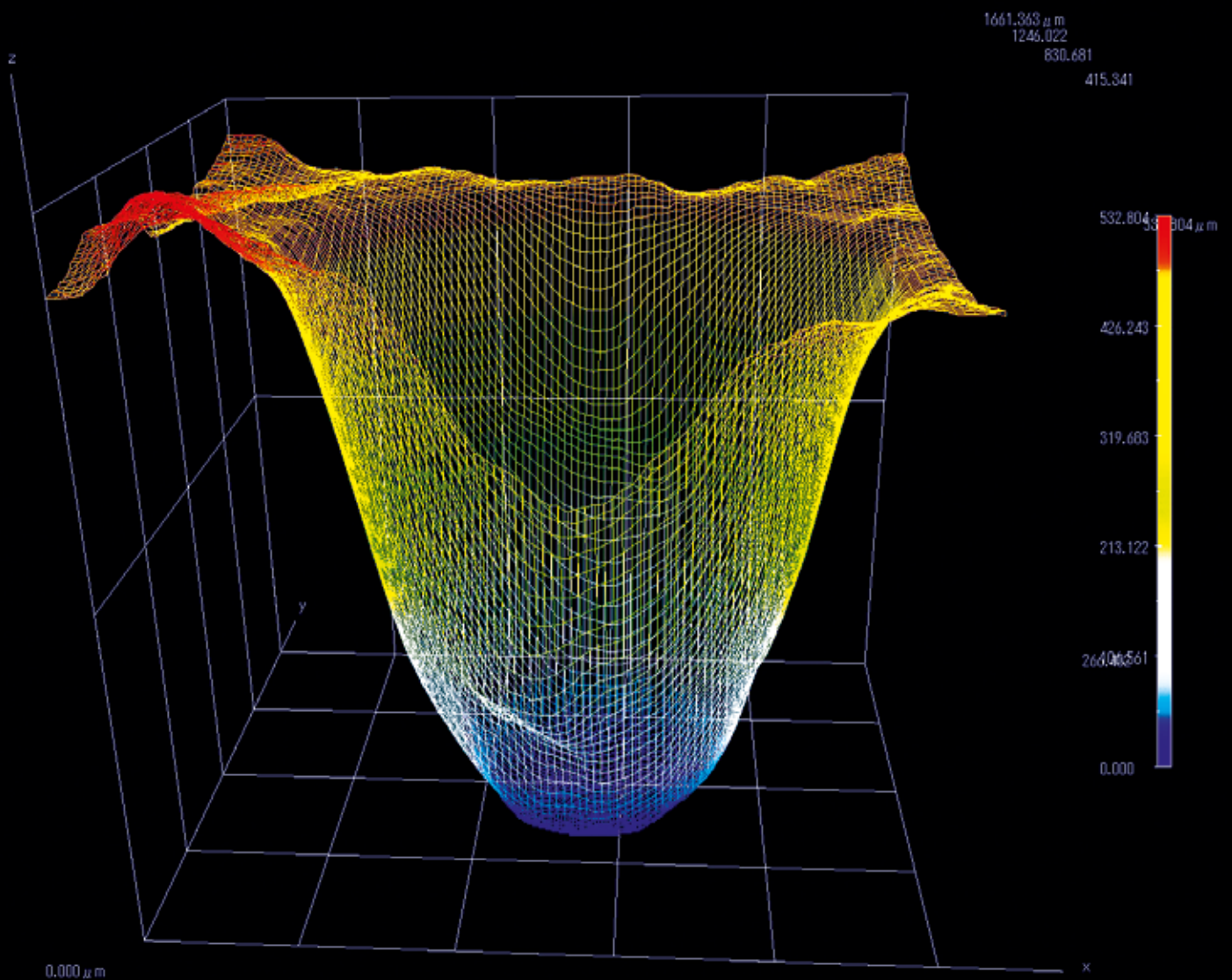
Semiconductor with **coaxial illumination**



Semiconductor with **directed coaxial illumination**

Maximum vertical resolution of Leica DVM systems

Zoom	Depth of field at Vmax	Vertical resolution
Leica VZ75 C @160x	250 μm	125 μm
Leica VZ80 C / Leica VZ80 RC @ 400x	80 μm	40 μm
Leica VZ100 @ 350x (10450392)	420 μm	210 μm
Leica VZ100 @ 700x (10450393)	110 μm	55 μm
Leica VZ100 @ 1400x (10450394)	4 μm	2 μm
Leica VZ100 @ 1400x (10450395)	3 μm	1.5 μm
Leica VZ100 @ 3500x (10450411)	1 μm	500 nm
Leica VZ100 @ 7000x (10450412)	700 nm	350 nm



Example from a real-world application

To illustrate this, let us provide an example from a real-world application. The fracture plane of steel in a tensile test is to be determined for quality inspection. The desired results are a qualitative representation as well as a quantitative statement about the surface. The desired vertical resolution is to be at least 120µm.

Here are the key optical and mechanical data of the equipment used:

- Optics used: Leica VZ80 RC, 8:1 zoom with magnification range 50 – 400x, at a maximum magnification of 400x
- Depth of field at maximum magnification of 400x is 80µm
- Resolution of the motorized focusing drive is 500 nm

In the example provided here, a theoretical vertical resolution of 40µm (depth of field / 2) is attained. The texture of the specimen to be examined has a high contrast range and can be distinguished easily by the software into sharp and out-of-focus areas. Therefore, the Leica DVM equipment configuration selected is outstandingly well suited for this application area and the required vertical resolution can be attained.

Resolution and application limits of Leica DVM systems

Based on the requirements of the specimen, it is easy to determine the suitable zoom optics. Usually, the criteria for the decision are the field of view and the lateral and vertical resolution. Generally speaking, the applications of Leica digital microscopy are in the microscopic and macroscopic area. For example, you want to examine an object field of 0.2 mm with a vertical resolution of 10 µm. From the technical data provided, you can tell that the Leica VZ100 with a 140x objective is suitable for this application. Additional help is provided by the Leica SmartTouch control unit based on a touch panel, which shows the specific depth of field being used on the display.

A challenge is presented by specimens that consist of transparent plastic, for example, and are to be captured in the microscopic range, i.e. with a digital magnification of greater than 1400x. The material provides only a little texture and the results will be unsatisfactory, even though the Leica DVM system is optically and mechanically suited to the task.

What alternatives do you have? For specimens with little texture, you can switch to another imaging method, such as confocal microscopy or interferometry. Leica DCM 3D, for example, combines both technologies in one instrument and is exceptionally well suited to specimens with little texture.

The table on the left provides an overview of the performance capabilities and application areas of the Leica DVM systems.

“With the user, for the user”

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• Life Science Division

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The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Surgical Division

The Leica Microsystems Surgical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

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