

Light Microscopy in Food Safety with Beer as an Example

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Authors: Erich Schuster, Holger Grawe
Bitburger Braugruppe GmbH, Germany

Dr. Thorsten Kern
Carl Zeiss Microscopy GmbH, Germany

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Figure 1 The wide range of beers
Courtesy of Bitburger Braugruppe GmbH, Germany

Introduction

Beer is one of the oldest process-engineered beverages known to humans. Enzymatic fermentation is followed by extraction. The microbial alcoholic fermentation gives it flavor and extends the shelf life. In terms of quality, both the great variety with regard to types of beer (Fig. 1) and the high sales volumes pose major challenges for breweries. Raw materials, storage conditions, and monitoring of the processes are just some such examples.

Furthermore, beer is also the subject of constant research and development. Nonalcoholic beers obtained by vacuum extraction after the actual brewing process and craft beers are typical examples of this.

Light microscopy plays an important role in process monitoring. The goal of microscopic examination of liquids in the brewery is the extensive and optimal visualization of typical particles. In addition, this modality is fast, efficient, and an integral part of the production process.



Figure 2 Monitoring of beer specimens
Courtesy of Bitburger Braugruppe GmbH, Germany

Its marked contrast and outstanding sensitivity have made darkfield imaging the modality of choice because the optical system detects visual impurities down the resolution limit (Fig. 4).

The fundamentals of brewing

During the first step of the brewing process, malted barley stored in silos is milled in a grain mill. This produces several parts leaving the husk of the grain intact (Fig. 3/1).

During mashing, the malt grist is mixed with water in the mash tun. The enzymes (amylases) in the malt grain are activated at different temperatures and cleave the insoluble starch into soluble sugars. This dissolves those substances in the malt that are important for the brewing process. (Fig. 3/2)

These enzymes are deactivated by the last temperature level.

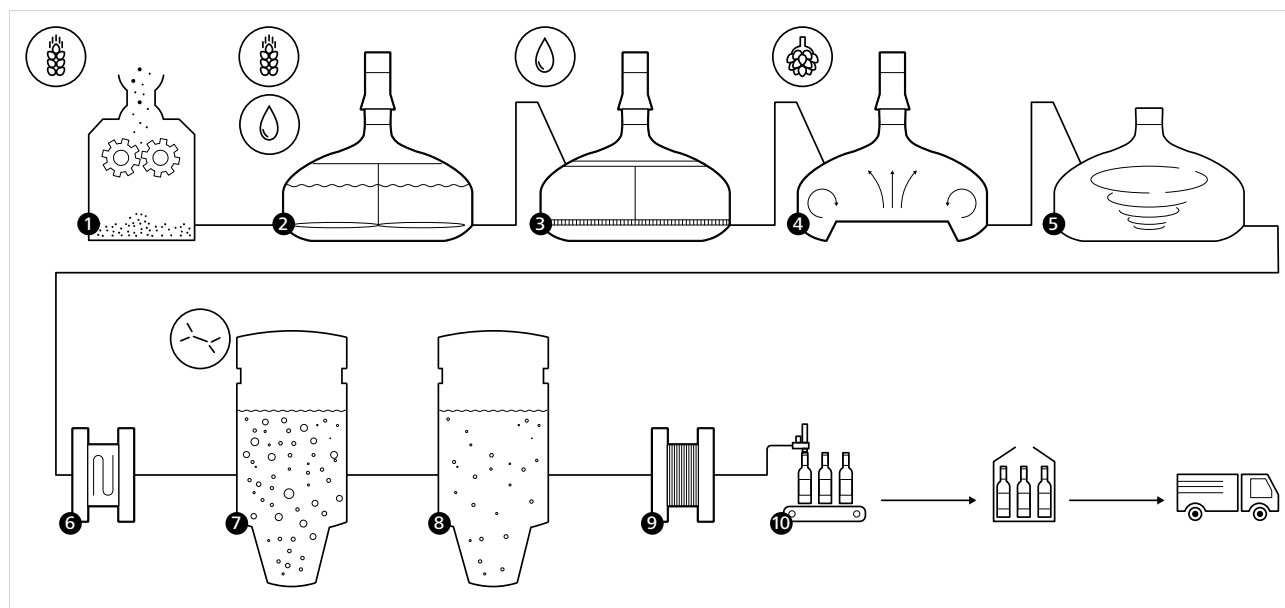


Figure 3 Brewing process

The next step in the process, called lautering, separates the solid constituents of the mash from the liquid. The solid constituents, known as draff or spent grain, remain in the lautertun and are sold as animal feed. The liquid, known as wort, containing all soluble constituents of the malt grains is pumped into the brew kettle or copper (Fig. 3/3).

Hops are added to the liquid in the kettle. The wort is then boiled for about one hour (Fig. 3/4).

The more hops are added, the more pronounced the hop flavor and the more bitter the beer will be later on. Depending on the type of beer, aromatic or bitter hops are added.

At the end of the boil, solid particles in the wort are separated out in the whirlpool (Fig. 3/5) before the wort is cooled down (Fig. 3/6) and the yeast added. Fermentation can start now.

In the fermentation vessels, the yeast will start the alcoholic fermentation process and convert the dissolved sugars from the malt into carbon dioxide and alcohol. At the end of this stage, the bottom-fermented yeast collects at the bottom of the fermentation vessels and can be siphoned off. The brewer now has young beer (Fig. 3/7).

The young beer is cooled down to temperatures around freezing and pumped into the storage tanks. It remains there for up to three months, depending on the type of beer. Storage allows the flavor of the beer to mature, cleans up undesirable ferment-

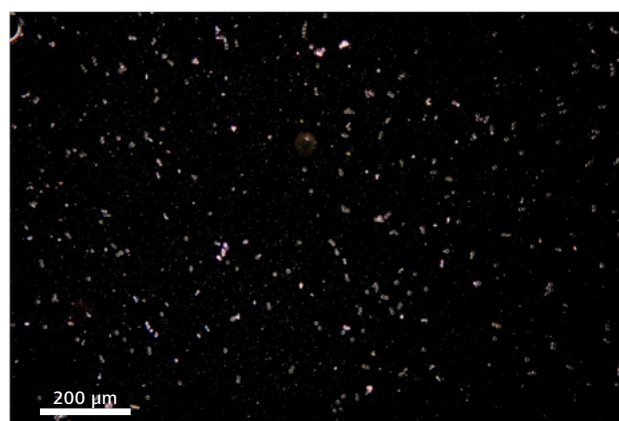


Figure 4 Brewing yeast in darkfield imaging; 10× objective
Courtesy of Bitburger Braugruppe GmbH, Germany

tation products, catabolizes any remaining sugars, dissolves carbon dioxide into the beer, and clears the beer through settling. (Fig. 3/8)

After storage, the beer goes through the filtration stage. Filtration removes any remaining trub from the beer. This then results in a clear, bright product known to the brewer as filtered beer (Fig. 3/9).

Once filtered, the beer is filled into bottles, cans, or kegs (Fig. 3/10).

Darkfield contrast imaging

A drop of beer to be examined is placed on a specimen slide and covered with a 0.17 cover slip. During the investigation,

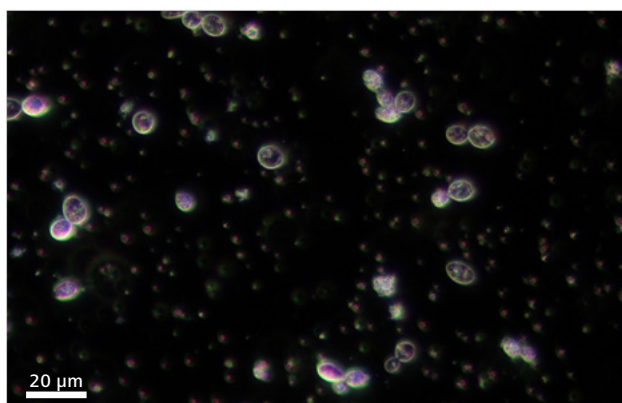


Figure 5 Brewing yeast in darkfield imaging, 63× objective
Courtesy of Bitburger Braugruppe GmbH, Germany

some of the liquid will evaporate or be squeezed out the sides of the cover slip, which will leave more and more particles in the same image plane (3D yeast agglomeration, particularly in the beginning).

Examination under the microscope is conducted using a 63× objective and corresponding darkfield condenser. Any particles will light up against the dark background (figs. 5 and 6).

When monitoring very different samples from all phases of the brewing process, sample origin is of great importance. Based on the correlation with a specific step in the process, it is obvious beforehand which characteristic image the dark-field imaging should demonstrate.

The investigator can therefore look specifically for any deviation from the micrograph. If such deviations are seen during the investigation, additional analyses can be initiated for identification.

For example, this may include living/dead cells for identification by fluorescent staining of microorganisms.

Recommended microscopy configuration

The configuration features of the microscope mentioned above are available in various optical packages. For example, the equipment ranges from the ZEISS Axio Lab.A1 with N-Achroplan 63× objective (# 420980-9900-000) and

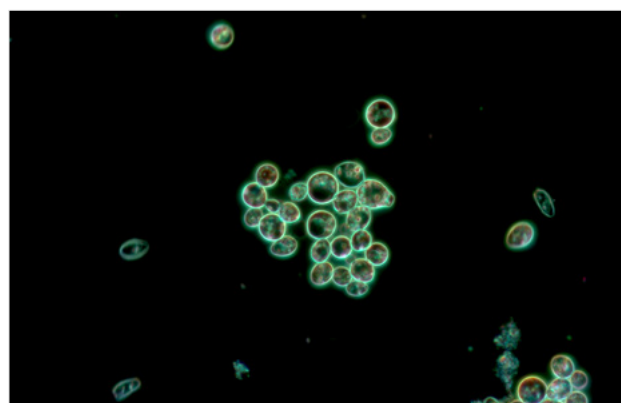


Figure 6 Brewing yeast in darkfield imaging, 63× objective
Courtesy of Bitburger Braugruppe GmbH, Germany

matching darkfield condenser for higher apertures (# 465505-0000-000 und 445323-0000-000) to the motorized Axio Imager with corresponding optical package. Depending on the application, the cameras will run not only with PC- but also WLAN-based systems – for example, AxioCam ERc 5s.

The ZEISS Primo Star HDcam (#415500-0059-000) with 40× darkfield slider (# 415500-1802-000) is also sometimes employed for basic brewing questions.

Conclusion

Darkfield microscopy is one modality for analyzing liquids in brewing. With its sensitivity and cost efficiency, this microscopy technique has proven itself in industrial settings. It allows high specimen throughput. Free and sometimes even non-OS specific software – for example, ZEISS Labscope – facilitates entry into documented quality control for small breweries as well.



Figure 7 Quality control during the brewing process
Courtesy of Bitburger Braugruppe GmbH, Germany

References:

- [1] „Technologie Brauer und Mälzer“ by Wolfgang Kunze, ISBN 3-921 690-31-5.



Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/microscopy



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