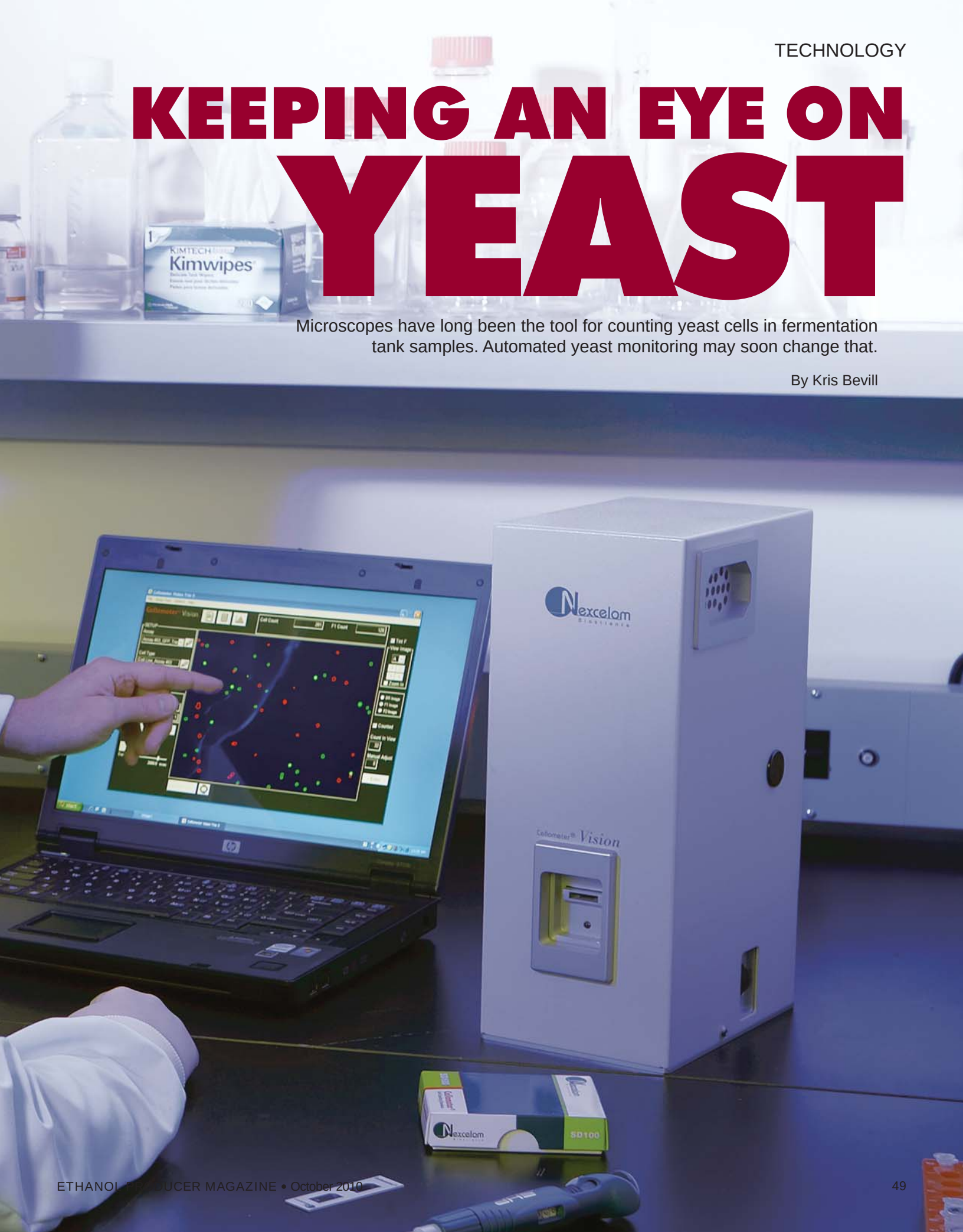
PHOTO: NEXCELOM BIOSCIENCE

KEEPING AN EYE ON YEAST

Microscopes have long been the tool for counting yeast cells in fermentation tank samples. Automated yeast monitoring may soon change that.

By Kris Bevill



In the current era of rapid technological advancements, it's hard to believe that the majority of ethanol plants still rely on a basic microscope to evaluate yeast cells. And yet, there they sit in almost every ethanol plant—remnants of a time before computers, when a skilled eye was the only method of determining how yeasts were holding up throughout fermentation. While microscopes are an effective instrument to count and evaluate yeast cells, are they still the best option for monitoring what can arguably be one of the most important components of ethanol production? Perhaps not for long. A handful of companies believe yeast monitoring methods are due for improvement and have developed products that could give ethanol producers a boost in keeping an eye on the fungi.

Yeast Activity Monitor

Naperville, Ill.-based Nalco Co. began developing its Yeast Activity Monitor a few years ago, according to YAM researcher and developer Michael Bradley. Nalco researchers sent prototypes to ethanol producers and brewers, most of whom were using microscopes to evaluate yeast activity, for first-hand feedback on the device's functionality. The company released the final product in May and has already installed the equipment at 10 to 15 U.S. ethanol plants and several outside the U.S., according to Bradley. The greatest appeal of the product is its accuracy when compared to microscopic monitoring methods. "It's not a subjective measurement," he says. "To put it simply, if you did the measurement or if I did it, we're going to get the same answer because there's nothing about your personal bias that's going to come into play, unlike a cell count. That's definitely a nice characteristic."

Each YAM system includes a touch screen computer that is connected to a digital balance and up to four probes. To use the equipment, a lab technician first weighs a sample from the plant's fermentation tank on the balance. The computer determines the amount of reagent required for the test and prompts the user to add the correct amount. Next, the technician inserts a probe into the sample and views the results on the touch screen monitor. Bradley says the entire measurement process takes approximately three minutes and is very user friendly. "One of the key things is that because you're doing it on a balance, it's very flexible," he says. "You can actually overshoot or undershoot the target [of reagent] that the computer gives you and it's perfectly fine because the computer will know how much you put in and will accommodate for that when it's calculating the result."

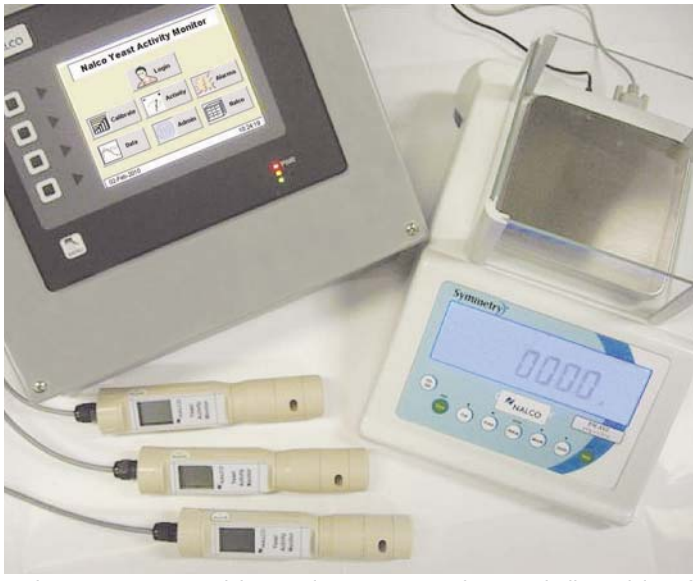


PHOTO: NALCO CO

Nalco Co.'s Yeast Activity Monitor measures the metabolic activity of yeast cells.

Bradley says YAM is unique because it doesn't count yeast cells at all. Instead, it measures the metabolic activity of the yeast cells in the sample. "There's a reason for that," he explains. "We think that this idea of counting cells and using a viability stain to classify the cells that you count as

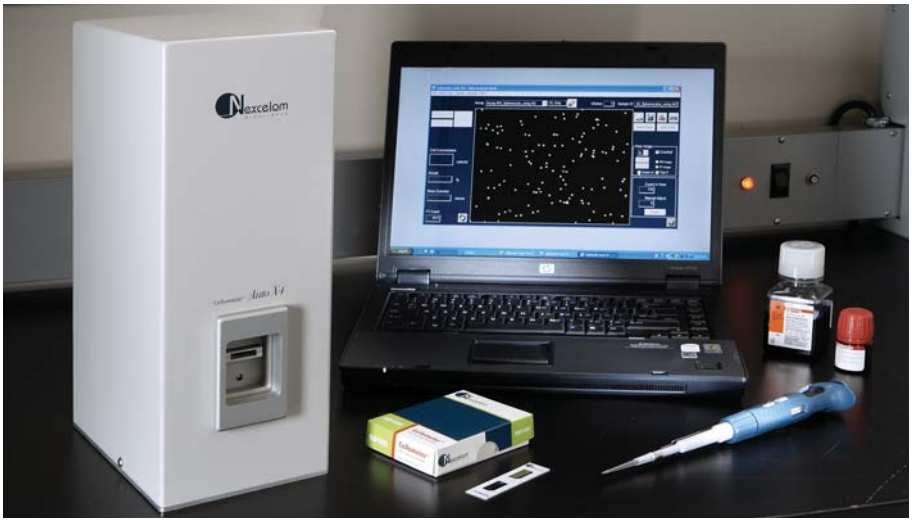


PHOTO: NEXCELOM BIOSCIENCE

Nexcelom Bioscience's Cellometer allows users to automatically count yeast and view images of the cell sample at the same time.

being either dead or alive is a limiting type of approach. We know from biology that cells are not just alive or dead. There's a whole scale of 'how alive are they?' And that's what you can't see with something binary like a viability stain. What we try to do is fill in that in-between space."

One of the benefits of looking through a microscope is that the user can evaluate overall health of the yeast cells, but Bradley says YAM can also detect stressed cells through a measured reduction in activity. "I would even argue that if your cells are stressed and you can't see it, and you don't know it, we would probably pick it up because we have a quantitative, non-subjective measurement that is a lot more in-depth than just 'what does the cell look like?' It really tells you what the cell is doing on the inside of the cell, where it actually does all of the work."

Cost of the YAM system varies depending on plant size, of course. Nalco estimates that a 100 MMgy plant would spend approximately $2,000 per month for reagents. Nalco provides the equipment to the plant at no cost, provided the plant purchases at least $1,300 of reagents per month. The equipment is a plug-and-play system, so total installation time is less than one hour and staff training can be completed in less than two hours, according to Bradley.

Cellometer

Instrument developer Nexcelom Bioscience LLC gears most of its equipment toward the life sciences industry, mainly in the areas of cancer research and drug discovery. But President and CEO Peter Li says the company began receiving inquiries from people in the brewery/winery/biofuel industries regarding cell counting and yeast viability a few years ago and decided to explore developing something useful to those industries. Developers soon discovered that everyone's yeast samples were very different, with corn mash samples being the most complex. "People asked if we could do it and, in the beginning, we couldn't," Li says. As a result of continued interest from customers and hands-on research conducted with ethanol plant and brewery samples, Nexcelom invented a special application for the Cellometer, which Li says provides reliable, consistent data from samples containing high amounts of debris, which is a typical characteristic of corn mash samples.

The Cellometer functions basically as a digitized microscope. It is an automated cell counting system that uses software to view sample images just as a person would. A technician places a fermentation sample into a disposable counting chamber and loads it into the Cellometer,

where the instrument captures an image, analyzes the data and creates concentration and viability results. Depending on the complexity of the cell sample, a reagent may need to be added to help the software identify live and dead cells. The entire process is completed in less than a minute according to Li, compared to an average time of 10 to 15 minutes for a microscopic count.

Li suggests the Cellometer would be a useful tool for producers who have microscopes but "are looking for a better solution." Because the software presents an image of the cell sample on a computer screen, the producer retains the ability to evaluate the cell sample with a trained eye, unlike other automated counters. "The user will always have the ability to interact and understand what the measurement results mean to them," he says. "It's different than if you use optical density, for instance, where you will never see the cells. In this case, you can see the cells, the uniformity of size, cells that have buds versus no buds, things like that."

Nexcelom's equipment package varies widely in price depending on the determined complexity of a customer's samples. Instrumentation costs, including software and a computer, range from $5,000 to $27,000 for the most complex sample analysis requirements. The reagent cost is relatively low at less than $1 per sample.

Another benefit of automated systems, according to both Li and Bradley, is their ability to record and store data. "If you're doing a measurement, I think it's important to get consistent data and have a good quality control record, and this will certainly do that," Li says.

"We thought data management was really lacking around the current practices of yeast handling," Bradley says. "There were people keeping notes on pieces of paper and sometimes those notes were getting transferred to a spreadsheet at the end of the month, but it was highly variable and you never knew what was going to happen. The biggest concern we had was that even if there was some attempt at data management, there was no attempt to actually proactively use that data during the process. We put a lot of focus on that while we were developing this product. We wanted it to be able to deliver the information real-time and to do it in a standard way."

The Best of Both Worlds

Chris Richards, global sales manager for Lallemand Ethanol Technology, says nine out of 10 plants his company works with still use a microscope to evaluate yeast activity. He sees advantages and disadvantages to both manual and automated methods and says the decision to use one method over another really depends on each particular plant's needs and staff. Microscopes are at a disadvantage in that it takes more time to complete a count and the accuracy of the count relies on the skill of the person looking through the scope. A skilled, experienced lab worker, however, may be able to detect certain anomalies when viewing cell samples that a machine could miss. And microscopes are very cheap. "The manual count is a bit of time and maybe 10 cents for the stain," he says. "If somebody has a well-trained team and they've got the time to do this, the manual cell counts are the most cost effective."

Machines, on the other hand, offer reliable, consistent measurements and produce results faster than a microscope method. Any automatic device is more costly to acquire than a microscope, but with some creative thought, plant managers can further utilize the equipment when making process control decisions or trouble shooting when optimizing fermentation.

"To be honest, the best solution is a blend of both worlds," Richards says. "As a plant manager, I would never want to lose that human interaction because it's always good to have someone looking at the yeast. If you don't look at yeast on a routine basis, when you do have a problem and you start looking at the

yeast you don't know what you're looking at." One course of action could be to conduct both manual and automatic tests on key parameters and run automated tests for the remaining items. "That gives you somebody looking at the cells each fermenter and it also gives you a manual calibration check against the machine," he says.

In late August, Illinois River Energy had just begun testing Nalco's YAM equipment at its 100 MMgy plant in Rochelle, Ill. Lab staff used a microscope to count yeast prior to testing YAM's automated equipment, and quality assurance lead Stephanie Brainard says they are interested in evaluating what an automated system might be able to provide in addition to a simple yeast count. "A yeast count is pretty cut and dry," she says. "It's either alive or dead or budding. This tells you the activity level of the yeast and is a pretty simple method." If comprehensive data can be gained that wouldn't otherwise be attained, Brainard believes the investment would be worth the price. "If it gives us a benefit and lets us see more of what's going on with our fermentation, I would say it's worth it," she says.

Validation

The National Corn-to-Ethanol Research Center trains plant employees for all aspects of ethanol production, including counting yeast cells. Sabrina Trupia, assistant director of biological research, says there's a definite learning curve when it comes to counting yeast and even when one becomes familiar with the process, there remains a large opportunity for error. "We currently in the lab have a very skilled staff, and they have spent many long hours perfecting their craft," she says. "In an ethanol plant, you maybe can't have people that are immediately that skilled at cell counting, so the counting method with a microscope is time-consuming and requires a lot of practice. We started looking at other ways and we know the brewing industry uses other ways of counting that are automated. Automated cell counters exist for bioscience. But basically, the problem in the ethanol industry is that we count cells in a medium that has its own chunks of other things. You have to recognize what's yeast and what are other things."

The NCERC began a research project in August to validate automatic counting methods, beginning with Nexcelom's Cellometer. In late August, Trupia told EPM the validation process was still underway but that early results were "really good." NCERC lab staffers enjoyed using the machine and she found it was becoming increasingly difficult to enforce manual cell counting in addition to instrumentation evaluations, a signal that the equipment was easily accepted by the users.

For all the differences—cost, subjectivity, ease and speed of use—the choice between a microscope and an automated system really comes down to the lab staff's ability to evaluate cell samples and/or the desire for comprehensive data. Accuracy is key. If a plant employs experienced lab staff who are skilled at counting yeast through a microscope and expect to stay employed at the facility indefinitely, then an automated system may not be worth the price. But, if accuracy is an area that could be improved within the lab, the initial investment required to purchase an automated system may pay for itself many times over in the long run. "If you don't have an accurate idea, you might end up making a decision that is expensive, wrong or detrimental," Trupia says. "The yeast is what makes it all happen, so it would be nice to know what it's doing at any given time. It's expensive to add yeast because you didn't count correctly. Maybe you didn't need to, or maybe there's a problem in the nutrients that need to be added, and so on. It's like any other mistake—the bottom line is affected." EP

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