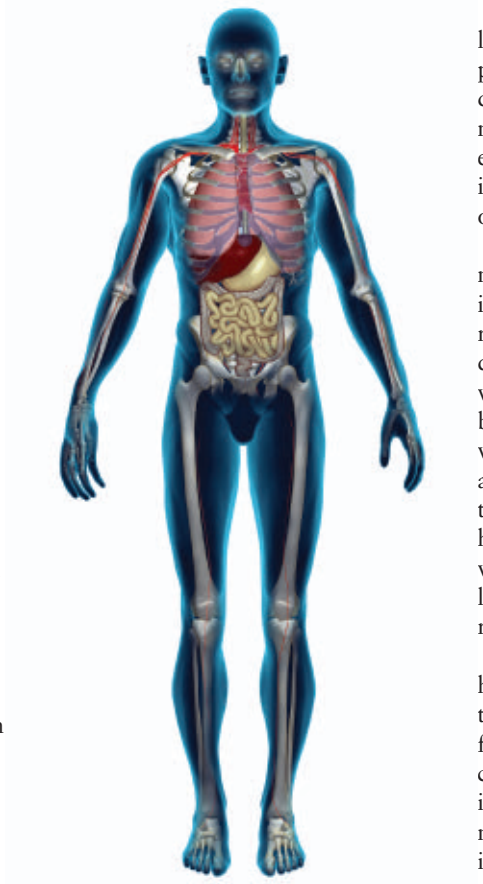
Automated Microscopy in Hypoxic Environments: *Does This Change Everything?*

Cutting-edge findings in the automation field spotlighted at SLAS

by Jessica Burdg, Contributing Science Writer

The overarching goal of laboratory automation—which, not so coincidentally, is shared by the Society for Lab Automation (SLAS)—is to leverage technologies for scientific advancement. At its annual conference held in January 2016, SLAS heard from a number of researchers who presented cutting edge findings in the field. Some, like Brian Rasnow, Ph.D., took it even further, taking automation to the extreme—extreme environments, that is.

Take, for example, a hypoxic chamber. As Dr. Rasnow explained in his talk, automated microcopy in hypoxic environments can yield substantially different results than similar experiments performed under O_2-saturated conditions. Let's examine what this means for life sciences as we know it and how it could potentially change the future of microscopy and even drug discovery.



In vivo cell interaction does not occur in ambient oxygen. Most organs exist with 2-14% oxygen.

Why Hypoxia?

Many cell biology results today are from experiments on cells grown in incubators under saturated O_2. Most mammalian cells, however, live with far less O_2 than is present in standard incubators. (For perspective, consider that the concentration of oxygen in the human lung is 20 percent while elsewhere in the body it is much lower.) What this means is that experiments conducted under more physiological conditions (i.e., in hypoxia) produce results with substantially different morphologies than those analyzed under normal conditions.

"As it turns out, all this cell culturing that we're doing today is highly unusual and artificial," Dr. Rasnow told *Laboratory Equipment*. "When we start looking at questions about things like metabolism where oxygen saturation plays a major role, then you have to really question whether studying mitochondrial metabolism in an artificially high oxygen environment will yield the same results as if you were repeating the experiment in an oxygen environment inside an organ."

As a result, some in the scientific community have called for many of the results in textbooks to be verified by experiments re-performed under hypoxic conditions–details like exactly what experiments and who will carry them out, however, haven't been outlined. Dr. Rasnow says it's not clear which conventional results will be validated and which will surprise us. He did state, though, that he would question any results having to do with the mitochondria or where oxidation states otherwise played a large role—which could be pretty much any result.

Why now, though? Why the focus on hypoxia at this particular scientific juncture? The re-energization may explain some failures in the drug discovery process. It's common knowledge that what is effective in the dish and even in laboratory mammalian specimens often fails to be effective in humans. One of the plausiblities is that researchers fail to replicate actual oxygen conditions in human tissues by growing cells in conventional CO_2 incubators. A hypoxic environment is a more appropriate way to mimic the environment within the human body, but it hasn't generally been done because it is difficult and dangerous–this is where automation comes in.

Automation is necessary when working in hypoxic environments because of the inherent dangers to researchers and their samples in these oxygen-depleted chambers. A large inhalation

of that atmosphere could render a human unconscious in a matter of moments, and suffocation is likely unless the person is physically removed from the environment within three to four minutes. Likewise, moving samples from a low oxygen to a normal oxygen environment can ruin their validity. When we start talking about technology that helps mitigate these risks, we start talking about not only automation but miniaturization as well.

A Dedication to Automation, a Focus on Miniaturization

Dr. Rasnow is no stranger to automation, having served a decade as a Principal Scientist in Research and Automation Technologies for Amgen. There, his group automated drug discovery and developed new bio detection platforms. Today, he is a lecturer at California State University as well as Chief Scientist/Co-Founder of Etaluma, a life sciences company focused on microcopy.

So, what's the correlation to automation? One major brainchild of Etaluma is the Lumascope, a compact, inverted fluorescence microscope that provides high-resolution images. It's small, simplified and extraordinarily useful to researchers working in hypoxic chambers—which, if experiments are going to need to be repeated en masse, will be a significant number of them.

"In hypoxic chambers in the old days, you had to have the eye piece of the microscope protruding and plaster your head against the side of the chamber, and that didn't work very well," Dr. Rasnow said. "Lumascopes are safer and more functional."

Lumascopes are small, fit entirely within hypoxic chambers and are USB powered. It's even possible to put the monitor on the inside of the isolator chamber as well for added ergonomics (but still see the digital image on the outside), which—according to Etaluma—is good news for cell biologists prone to spending long hours on the bench.

Simplification and miniaturization are additional key life science trends that fall under the laboratory automation umbrella. In cell biology, for example, hypoxic chamber real estate is expensive. Having automated, smaller microscopes (like Lumascopes) means more room for cells, reagents and other equipment—but there's more to it than that.

"On the automation side, I envision the industry will lean heavily into miniaturization," Dr. Rasnow told *Laboratory Equipment*. "Miniaturization of the apparatus and miniaturization of the assay format so you can achieve the high throughput with smaller volumes to better create and work in the oxygen depleted atmospheres."

Doing hypoxic screening in smaller formats may push researchers to move on from microtiter plates to closed microfluidic chips. It will become unnecessary, then, for large chambers or entire rooms to be used to screen what can be done entirely inside a hand-held piece of plastic.

The Takeaway

Does it matter that cells have different morphologies when examined in hypoxic environments than they do when analyzed using more traditional techniques? Maybe. Even if not, though, a major takeaway from this conversation is that automation is a game-changer for the future of microscopy, especially as it relates to drug discovery. More so an issue of differing functions than differing morphologies, and phenotypes are much harder to view in a dish or microtiter plate.

"This certainly raises some questions about what we've been doing thus far [in drug discovery]," Dr. Rasnow told *Laboratory Equipment*. "If I was running a pharmaceutical pipeline, I'd invest in repeating key experiments in hypoxic chambers before going to expensive human trials that cost up to tens of millions of dollars."

The LS620 with Phase Contrast Accessory. Credit: Etaluma