FEBRUARY 2018 # 39

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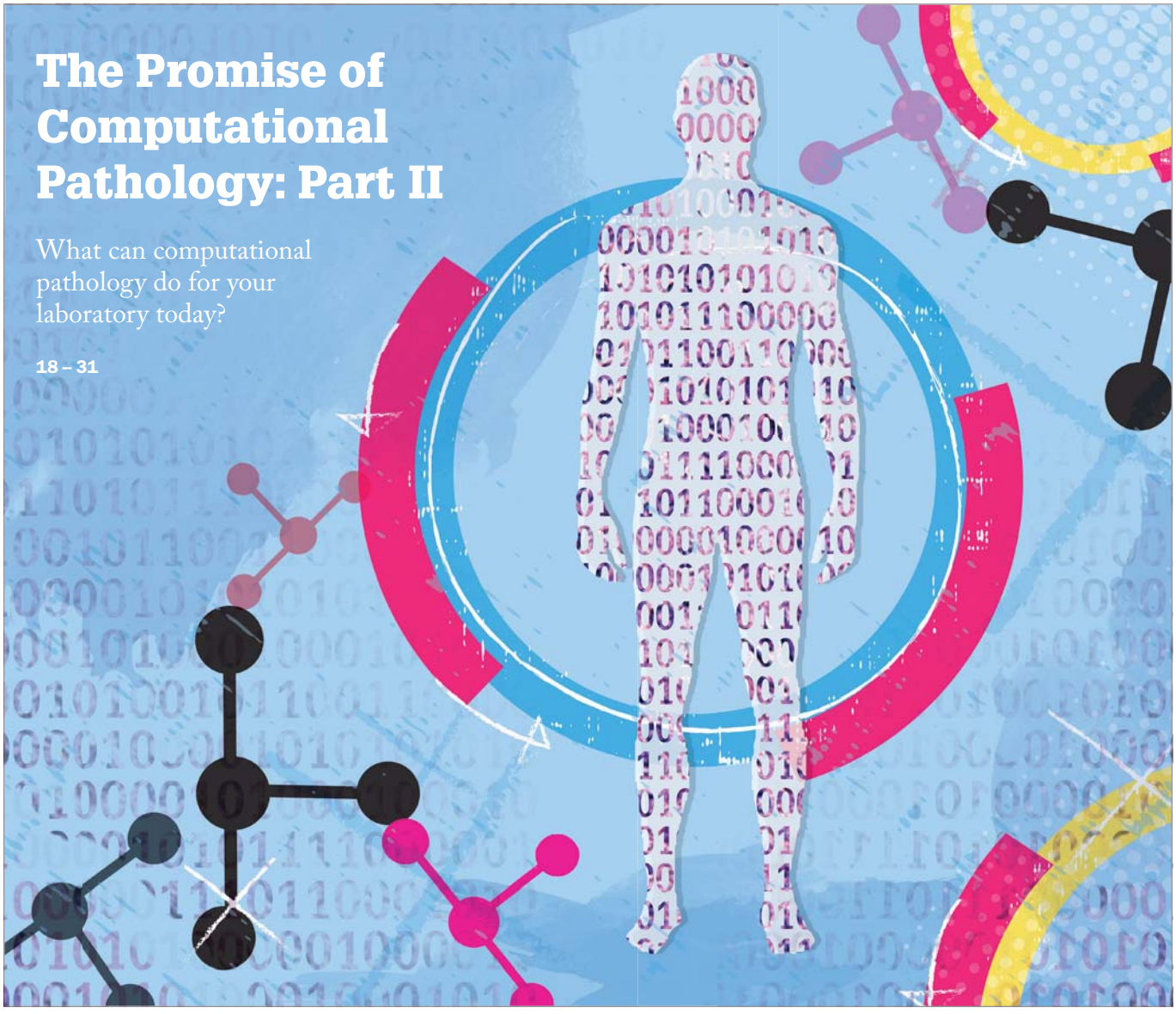

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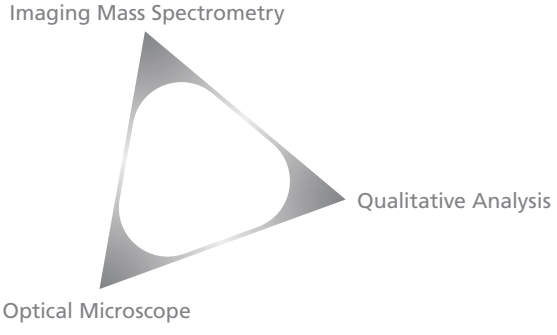

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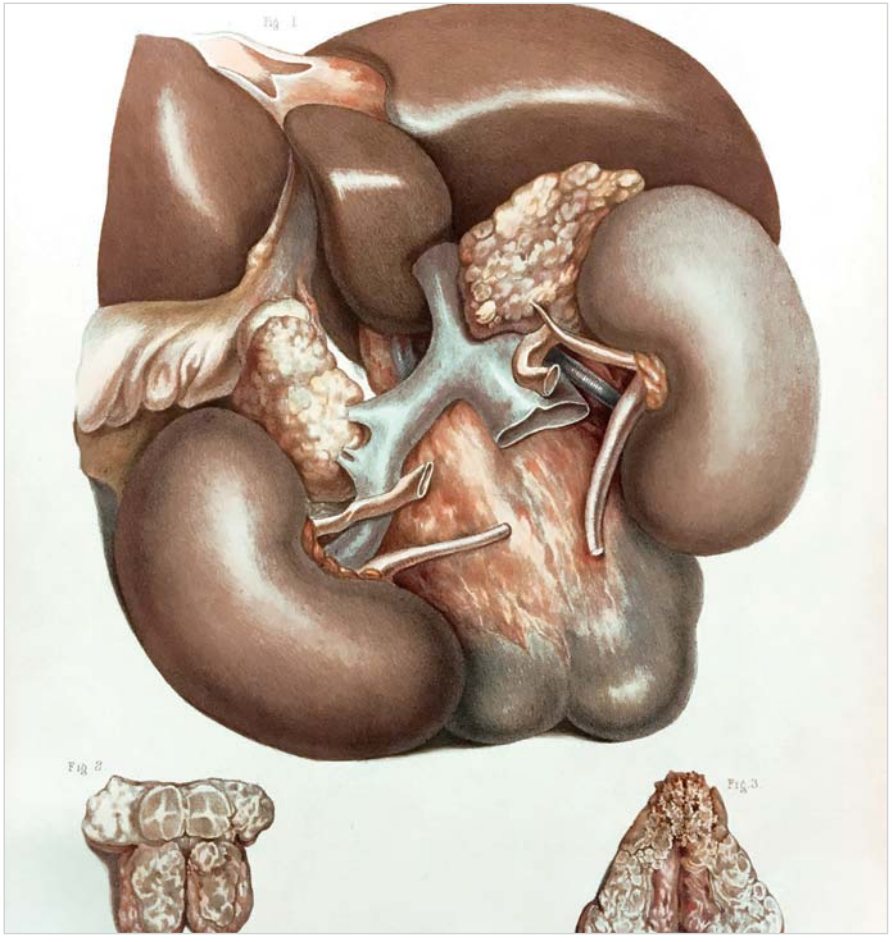
Case of the Month

In this figure from Thomas Addison's classic paper, one can clearly see that the adrenal glands are pathologically altered. What was the cause of adrenal failure in this patient suffering from Addison's disease?



a. Autoimmune adrenalitis

b. Tuberculosis

c. Septicemia with hemorrhagic necrosis of both adrenals

d. Adrenal atrophy of unknown cause

To register your guess, please go to http://tp.txp.to/0218/case-of-the-month
We will reveal the answer in next month's issue!

Answer to last issue's Case of the Month…

B. Intestinal atresia

Intestinal atresia is a loss of continuity of the intestinal lumen, presumed to be due to an intrauterine insult or a developmental abnormality. It most commonly affects the small bowel and only rarely the large bowel. Patients usually present very early in life with abdominal distension, vomiting, and failure to pass meconium. There is a high degree of association with other congenital abnormalities.

Submitted by Laura Brown, The University of Kansas School of Medicine, Kansas City, USA.

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An artist's representation of the influence of computational pathology on laboratory medicine and how digitization increasingly affects every step of the diagnostic process.

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the Pathologist
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Reading Between the Lines

Are your scientific papers and medical reports accessible to those outside your laboratory?

As an editor, it's my job to ensure that text is not only correct, but also clear and readable – and, ideally, fun to read as well. It's an instinct that I can never entirely switch off, so I often spot instances of unclear wording and unnecessary jargon in everyday life. And that, of course, includes the scientific literature I read. Could that sentence have been shorter and more approachable? Was that piece of specialist language really necessary – and, if not, has it only made the paper harder for non-specialists to understand?

Questions like these are growing increasingly relevant in the modern scientific world. Even specialty subjects often require large-scale collaboration to produce high-impact results. Genetics is a good example; whereas previously a graduate student could spend years creating a custom cell line, publish the work in Nature, and graduate, that work is now only the first few weeks of a much larger project – one that may require the assistance of biochemists, microscopists, bioinformaticians, statisticians, medical specialists, and many others. But if the statistician – trained extensively in mathematics, but less so in the life sciences – can't understand your experiments, they may struggle to contribute to the best of their ability. If the interdisciplinary scientist who reads your paper doesn't understand what you've done, how can they take the next step in the research process?

A recent study in eLife showed that scientific texts are becoming steadily less and less readable, with longer words, longer sentences, and more jargon (1). The trend may be in part because science itself is becoming more sophisticated, but complex concepts can be explained with simple but powerful prose – something we strive for in the pages of The Pathologist.

Do your papers suffer from low readability? Are they inaccessible to your colleagues or other medical professionals? How about your institution's lawyers, accountants, or maintenance staff? It may be worth taking a second look at the text. The same applies to medical reports – after all, not every clinician has a high level of laboratory medicine expertise. And perhaps your patients themselves may even want to read your reports to learn about the role you play in their treatment. Wouldn't it be nice if they could understand at least some of what you wrote?

Michael Schubert
Editor

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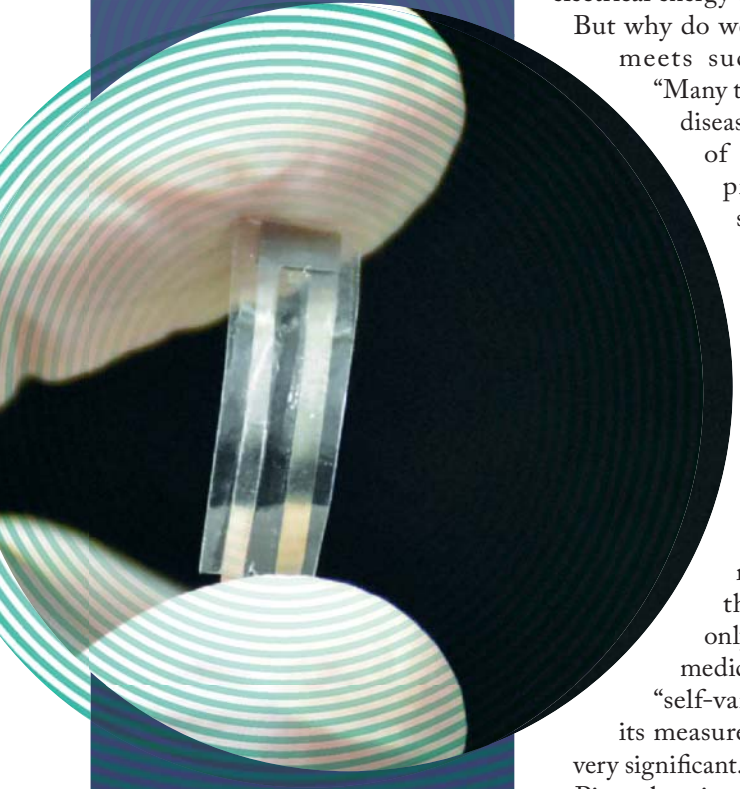
Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

Email: edit@thepathologist.com



Here Today, Gone Tomorrow

A movement-powered, biodegradable sensor aims to monitor vital pressures in the body

"The ultimate goal is to improve patients' quality of life," says Thanh Nguyen, Assistant Professor of Mechanical Engineering at the University of Connecticut. To that end, he's worked with colleagues to develop a biodegradable piezoelectric sensor that measures vital physiological pressures (1). The 5 mm by 5 mm sensor, only 200 µm thick, is made of flexible biocompatible materials, some of which generate electrical energy through deformation.

But why do we need a device that meets such specifications? "Many traumatic injuries, and diseases lead to the buildup of dangerous internal pressures." Nguyen says. "Monitoring them is tremendously important to provide timely intervention. The available sensors to monitor those pressures, however, are often bulky and non-degradable, requiring invasive removal surgery. We think a sensor based only on commonly used medical materials that can "self-vanish" after finishing its measurement task would be very significant."

Piezoelectric devices offer several advantages over existing bioelectronic sensors. "First, [our device] is very biocompatible because it is based on a common medical material (PLLA) that has been used for FDA-approved surgical sutures, bone scaffolds, and drug delivery devices. Second, it self-emits electrical signals when subjected to force, potentially avoiding the use of a battery in conventional implanted medical sensors. And finally, the sensors could use that self-emitting signal to produce useful electrical stimulation for tissue healing and growth," says Nguyen. Applications for the sensor could range from measuring diaphragm and transpulmonary pressures to monitoring intraocular and intracranial pressures in glaucoma and hydrocephalus, respectively.

But if the sensor is degradable, how long is it able to provide useful readings? Nguyen's laboratory implanted several of the devices into mice, then tested and confirmed the viability of the sensors for up to 16 days – for context, that's four times longer than the typical length of time for monitoring intracranial pressure. They also put the device through an accelerated degradation process (at 74°C) and found that the sensor completely broke down after 56 days. If necessary, though, its lifetime can be reduced or extended by altering the thickness of the device and the molecular weight of the materials used. Indeed, further development aims to create a biodegradable sensor that gives the user robust control over its lifetime.

Nguyen is hopeful for the overall future of body-bound sensors, but points out a few essentials: "Implanted sensors would have to be multifunctional, small, flexible, wireless, self-powered, and especially biocompatible, in the sense that all byproducts from the degradation process should be safe for long-term use inside the body."

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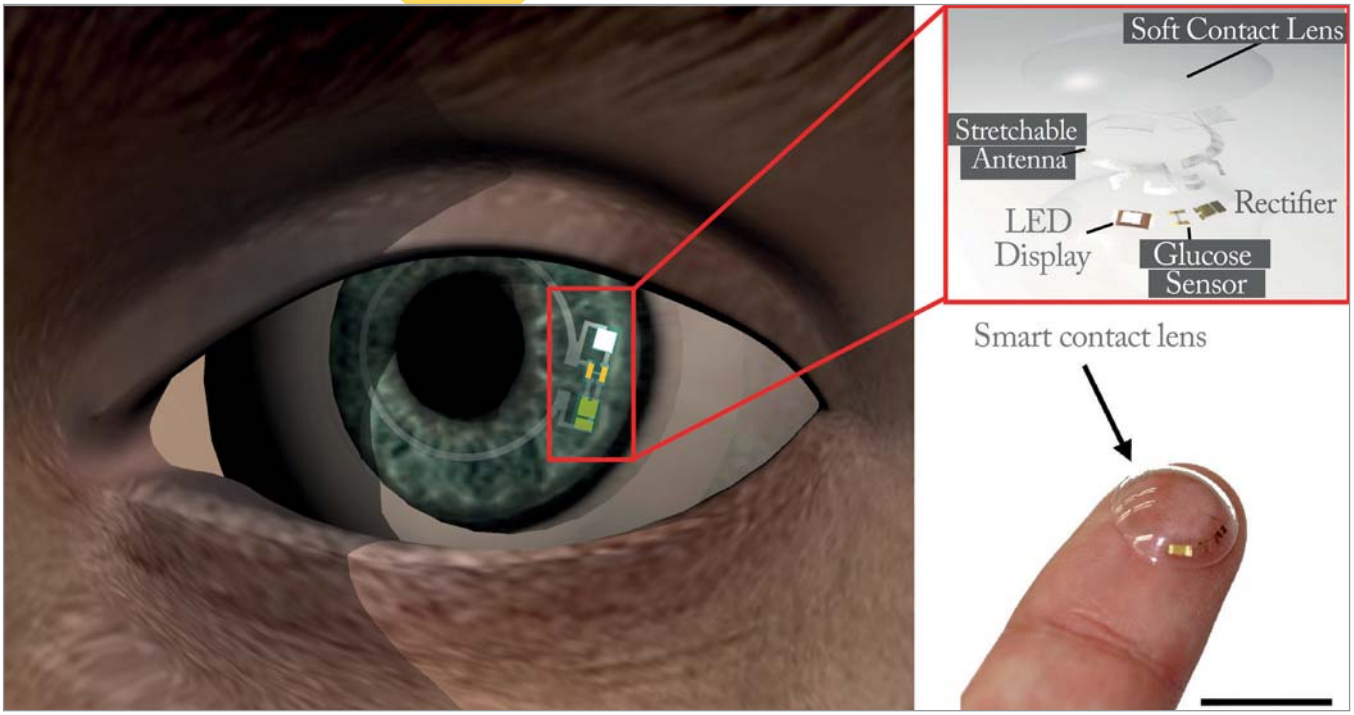

Figure 1. The soft, smart contact lens is comprised of a hybrid substrate, functional devices (rectifier, LED, and glucose sensor) and a transparent, stretchable conductor (for antenna and interconnects). Electric power for the LED pixel and glucose sensor is wirelessly transmitted to the lens through the antenna.

Sweet Tears

A noninvasive method to monitor disease in the eye

Many patients with diabetes would be happy to see the back of their blood glucose monitors and the daily finger prick tests. Enter a team of scientists from the Ulsan National Institute of Science and Technology (UNIST), South Korea, who have created a means of wirelessly monitoring glucose levels with a soft contact lens.

"Embedded within our smart contact lens are electronic circuits, an antenna, a glucose sensor, and LED pixels integrated as stretchable forms," explains Jang-Ung Park (1). "This improves the comfort and wearing-time of the lens compared with previous smart lenses that were hard due to having brittle and more rigid components."

Their sensor comprises a graphene surface to which glucose oxidase (GOD) enzyme is immobilized. Glucose-containing tears pass through the sensor channel. GOD oxidizes the glucose, which releases electrons in a concentration-dependent manner that the sensor detects, enabling determination of the glucose concentration. The sensor contains an LED that responds to changes in resistance (which are coupled to tear glucose concentration). Below 0.9 mM, the LED emits light; above this, the LED pixel turns off, providing a visible cue that the glucose threshold has been reached (Figure 1).

So far, the team have demonstrated that the device can respond to changing glucose concentrations in rabbit eyes, and they plan to move into clinical tests in humans. But might it have further diagnostic applications? The researchers write that their novel system could "provide a platform for wireless, continuous, and noninvasive monitoring of physiological conditions, as well as the detection of biomarkers associated with ocular and other diseases."

Reference

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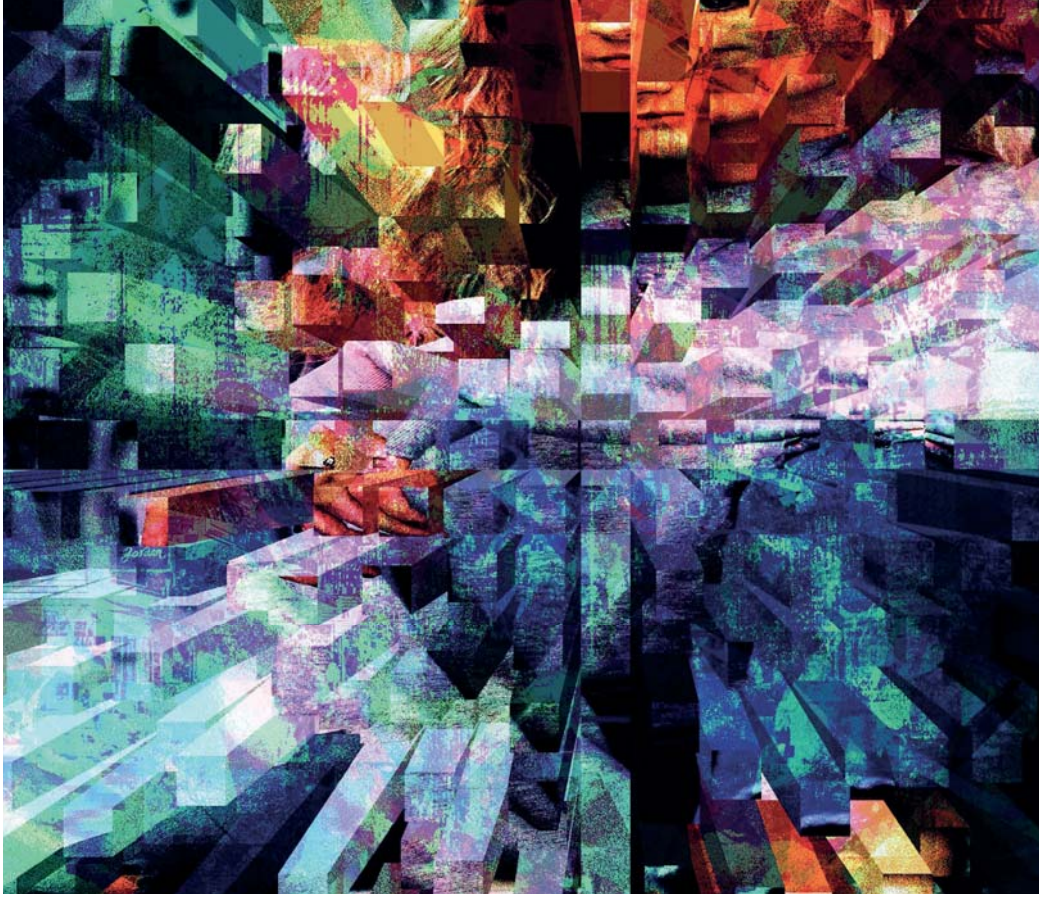


Pixel Perfect

A spatial gene expression technique for improved tissue sample analysis

Existing gene expression analysis tools, though accurate, are slow and cumbersome to use. Is this trade-off really necessary? Not according to a multidisciplinary group of researchers who incorporated the best of both worlds into a technique that performs on-chip tissue analysis in under two hours: pixelated RT-LAMP (reverse transcriptase loop mediated isothermal amplification).

To find out more, we spoke to Anarup Ganguli, first author of the resulting publication (1) and research assistant at the University of Illinois at Urbana-Champaign, USA.

Why investigate gene expression analysis?

Gene expression analysis has many applications, such as revealing the molecular basis for developmental processes in organisms or helping us to understand the role of differential gene expression in normal and disease conditions. For tissue samples, the spatial localization of gene expression can unravel important insights into tissue heterogeneity, functionality, and pathological transformations – but the ability to maintain this spatial information remains an enduring challenge in tissue sections routinely used for pathology. This very challenge prompted us to develop a simple, rapid, quantitative technique to analyze the spatial distribution of nucleic acid targets across a tissue section.

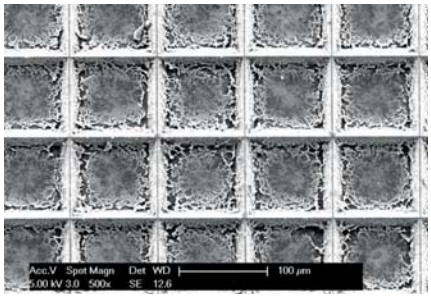
What does your technique entail?

Our pixelated RT-LAMP approach uses parallel on-chip nucleic acid amplification reactions to provide the distribution of target sequences directly from tissue, without the need for analyte isolation or purification. We do this on a fingernail-sized chip with an array containing more than 5,000 picoliter-volume wells.

We anticipate that this technique, with its ease of use, fast turnaround, and quantitative molecular outputs, will become an invaluable tissue analysis tool for researchers and clinicians in the biomedical arena.

What were the greatest challenges?

The biggest hurdles were automating the tissue microdissection to cut the tissue into 100-micron pixels while preserving the spatial location of each pixel – a process we call "tissue pixelation," loading more than 5,000 wells with 175 picoliters of amplification reagents, and optimizing the protocol to make the RT-LAMP amplification reactions happen in the presence of the tissue debris. In other words, it was all challenging!



What does the future hold?

We hope to use our technique to map genetic mutations in lung, breast, and prostate cancers. We will also work to reduce the size of the wells on the chip below the current 100 microns, which would allow us to examine individual cells at a higher resolution.

With the reactions and biosensing now occurring in picoliter and even lower scales, portability comes as an inherent advantage. However, the true advantage of our technique is the ability to look at the nucleic acid composition of a large region of tissue with high spatial resolution – something no other existing technique can do.

Reference

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Super Saliva Test

Ultrasensitive PCR powers an oral fluid assay for earlier HIV diagnosis

The advent of antiretroviral therapies to control HIV and the use of pre-exposure prophylaxis has turned a once-terminal illness into a relatively manageable disease when caught early enough. But although great strides have been made to curb the virus, 36.7 million people worldwide currently live with HIV – nearly half of whom are unsure of the status of their infection (1). Clearly, without access to adequate testing, it's difficult for both patients and the medical community to keep track. And that's why Carolyn Bertozzi and her team decided to develop a highly sensitive HIV assay for saliva (2).

At the moment, there are two main types of tests for screening HIV, each with pros and cons: blood tests are highly sensitive, but have a poor rate of compliance despite being minimally invasive; oral fluid tests, on the other hand, although noninvasive, typically suffer from poor sensitivity because of the lower concentrations of anti-HIV antibodies. The new oral fluid assay addresses the challenge by using antibody detection by agglutination-PCR (ADAP) – making it 1,000–10,000 times more sensitive than existing tests. "ADAP is based on the concept of proximity ligation PCR, which we knew had the capability of ultrasensitive DNA detection. This ADAP was designed to bring the sensitivity of PCR to the problem of antibody detection," says Bertozzi, lead investigator, and Anne T. and Robert M. Bass Professor of Chemistry at Stanford University. "The ADAP test can enable earlier detection of HIV infection in the context of population screening using oral fluid, which is easier to collect and far less risky for healthcare workers because, unlike blood, oral fluid is not infectious."

Bertozzi acknowledges that translating the research to the clinic is no small feat: "The biggest hurdles that lie ahead are larger studies with longitudinal data to judge how ADAP compares with current oral and blood-based tests." But the team also see great promise in the ADAP assays, and plan to develop analogous tests for other infectious and autoimmune diseases that produce autoantibody biomarkers.

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Brightening the Forecast for Skin Cancer

Can ctDNA blood levels predict the risk of relapse in melanoma patients?

Despite many cases being preventable, melanoma remains one of the most common cancers. Patients with resected stage II or III lesions often develop distant metastases – but current methods cannot accurately predict who is most at risk.

Rebecca Lee, a research associate at the University of Manchester, is first author on research that investigates the predictive potential of circulating tumor DNA (1). We spoke with her to find out how genetic testing using ctDNA could improve patient care.

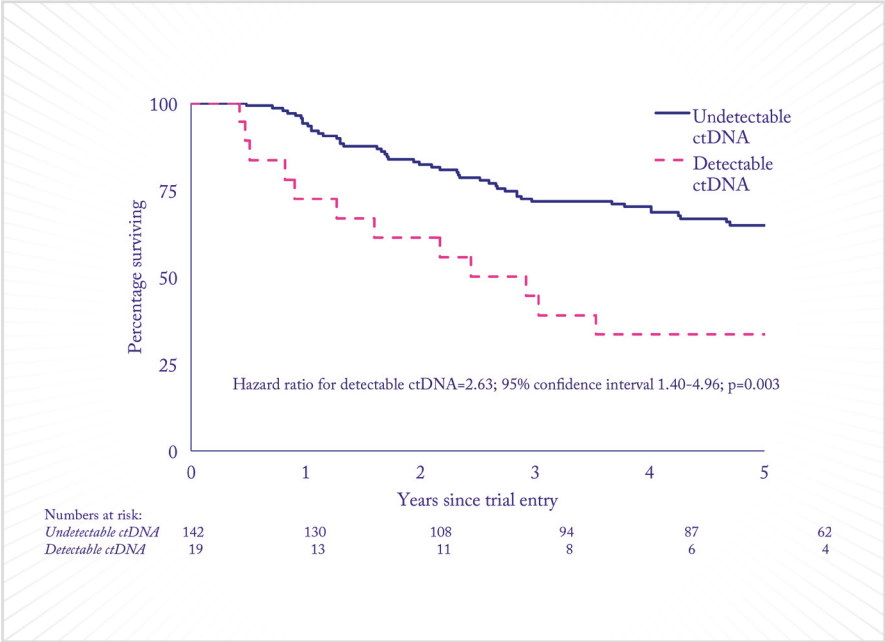
What main advantage does your approach offer?

Current methods use a combination of clinical and pathological features – mainly based on the American Joint Committee on Cancer (AJCC) staging – to predict recurrence. However, they are limited in their ability to accurately predict relapse for each individual patient. It is of great importance to identify those patients at highest risk of relapse, especially in the era of effective adjuvant therapies for melanoma. We believe that genetic testing using ctDNA detects minimal residual disease following surgery and, therefore, can be used in conjunction with AJCC staging to identify high-risk groups.

What could this test mean for skin cancer patient care?

We believe liquid biopsies can improve care in several key areas. The first is early detection of micro-metastatic relapse in patients who have had surgery performed with curative intent as part of secondary prevention strategies. The second is the monitoring of tumor burden; for example, in patients receiving treatment for stage IV disease, it could be used to detect early progression of treatment. Finally, it can be used to identify mutations associated with either a response to therapy (such as *BRAF* mutation/*BRAF* inhibitors) or a resistance to therapy.

We are currently developing clinical trials to test whether early treatment based on detection of ctDNA could improve outcomes for patients, so we



would expect it to become available in the clinic in about five to eight years' time.

How did you approach development?

As a proof of principle, we decided to examine *BRAF* and *NRAS* mutations, as these account for approximately 80 percent of driver mutations in melanoma. We could also look at other mutations, which would require extra steps – first, sequencing the tumor to identify the mutations, then analyzing the ctDNA in plasma. We decided to use droplet digital PCR (ddPCR) – a clinically relevant technique because it is relatively fast and inexpensive compared with next generation sequencing (NGS) approaches. In addition, bioinformatics support is not required, which makes it easier to translate into the clinic.

How sensitive is the test?

Sensitivity for predicting relapse was 18 percent and specificity was 95 percent; if ctDNA is detected, there is an extremely high chance that the patient will relapse. A lack of detection, however, does not necessarily mean that the patient will not relapse – although it becomes more likely after a longer period of time. We feel that longitudinal monitoring of patients will improve the ability of the test to pick up micro-metastatic relapse.

How would the test fit into the existing pipeline?

The majority of the processing is relatively straightforward and can be automated. The process is already used to detect certain mutations – for example, *EGFR* mutations in lung cancer – so it would be a question of building the capacity into the health system to perform more tests, and establishing pipelines whereby samples are sent to the laboratories in a timely manner, just like other blood tests. We see it as an additional tool to aid in the diagnosis and monitoring of the disease.

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In My View

In this opinion section, experts from across the world share a single strongly held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of laboratory medicine. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@thepathologist.com

Move Out of the Tunnel… and into the Light!

When it comes to glaucoma care, metabolomics could well be the future

By João Barbosa-Breda, Glaucoma Fellow, Ophthalmology Research Group of the KU Leuven (University of Leuven), Belgium

I believe metabolomics has the potential to shed light into the pathophysiology behind glaucomatous damage, as well as providing potential biomarkers for early glaucoma detection.

Metabolomics is the detailed study of metabolites created by the cellular processes in an organism. Influenced by both genetic and environmental factors, the metabolite profile gives us a "fingerprint" of the health status of the organism at the time of sampling. But aside from disease mechanisms and biomarkers, this technology has also been increasingly used for drug target discovery and the prediction of drug effects – and it can help move medical care towards a more personalized approach.

For all of these reasons, metabolomics is becoming widely used across different fields, from cancer research to obstetrics and even ophthalmology, where it keeps proving its value. A recent paper has shown the potential of metabolomics for the diagnosis of age-related macular degeneration (AMD); both the presence and stages of the disease could be distinguished by identifying differences in metabolic profiles in controls and patients with AMD (1). Glaucoma, the world's leading cause of irreversible blindness (2), is also a good candidate for metabolomic analysis; it has the potential to break knowledge barriers, driving us toward new treatments and personalized care. Our research group, led by Ingeborg Stalmans, recently reviewed results that have already been delivered by metabolomics in terms of understanding the pathophysiological processes of glaucoma in a clinical setting (3). Only one metabolome-wide study for glaucoma has been published to date; it identified significant metabolic differences in blood plasma samples from patients with primary open-angle glaucoma (POAG) and samples from healthy controls (4).

"Metabolomics will help bring glaucoma care into a new era."

We feel that we can push this research a step further, so we are collecting blood, urine and aqueous humor samples from patients with POAG who are undergoing surgery (MISO Study; NCT03098316). All samples will be analyzed using two complementary metabolomics techniques (mass spectrometry and nuclear magnetic resonance spectroscopy) and compared with control samples (from patients with

cataract and no other eye disease). As metabolomics provides an overview of whole body metabolism, we are also including a study group of patients with normal-tension glaucoma, because systemic vascular dysregulation is thought to play an important role in the pathogenesis in this group of patients.

In my view, metabolomics will help bring glaucoma care into a new era by speeding diagnosis, reducing the numbers of patients arriving in our office already at an advanced stage of disease, and revealing new research paths that can be used to develop new drugs and improve ophthalmic care.

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A Blip on the Radar

Why I'd like to thank the pathologist who turned a potentially life-threatening disease into a single day of treatment

By Susan Schubert, Registered Nurse/ State Registered Nurse, Edmonton Urgent Services Team, Alberta Health Services, Edmonton, Canada

It was easy to see the spot. When the doctor showed me the chest X-ray, he didn't really need to point it out to me. And that's why it was all the more difficult to understand how it had been missed the first time…

I'd already had an X-ray the year before as part of a routine medical examination when I was living in Austria. (My husband worked for the Embassy, and the medical coverage was good – one of the benefits). Although in retrospect the spot was clearly visible, the local physician told me that there were no abnormalities.

A year later, we moved back to Canada and I resumed my work as a nurse. A routine medical examination was a prerequisite for employment, so – among other things – I was given another chest X-ray. The spot was seen immediately – but this time, it was flagged and discussed with me. What was it? Why hadn't it been addressed previously? One possibility the doctors considered was tuberculosis, which meant that my employment was immediately suspended to prevent its potential spread. Further testing ruled out that disease, so we began to investigate other possibilities.

Eventually, I was told that the spot was likely a tumor. My options were "watchful waiting" (monitoring the spot to see whether or not it would develop further) or to have a biopsy, with surgery to follow if needed. I was in good general health and fairly fit, and the spot itself was small (about the size of a dime) and discrete, so I opted to have an X-ray guided biopsy performed under sedation.

As a nurse, I knew what would happen to my sample next; it would be sent to pathology, where a diagnostic professional would take a close look and determine my next steps. Would I need watchful waiting? Surgery? Additional treatment? Thankfully, as a healthcare professional, I knew I could rely on my laboratory-based colleagues to guide my medical care.

> *"As a healthcare professional, I knew I could rely on my laboratory-based colleagues to guide my medical care."*

In this case, the pathologist speedily diagnosed a carcinoid tumor – a rare, slow-growing cancer that was unlikely to spread, but that would need to be removed as soon as possible. Armed with this information, a surgeon proceeded to remove the tumor under full anesthetic. He cut out a generous wedge of my right lung as well – so generous, in fact, that no follow-up radiation was required. The result was a single afternoon of surgery and a few weeks of concern and recuperation, after which I was able to

resume being a wife, the mother of two young children – and a nurse. Of course, I still had several years of follow-up appointments for scans, tests and check-ups, but my cancer no longer had the power to disrupt my life or prevent me from living it fully.

It's the ideal outcome – a long and happy life after a potentially devastating diagnosis. But something was missing: as both a patient and a professional, I would have appreciated the opportunity to speak to my pathologist directly. It would probably have made no difference to my treatment, but I would like to have learned more about my tumor. The surgeon was a bit inarticulate and only felt he had to tell me it was "a type of cancer"; evidently, he felt I didn't need to know more. However, I would have liked to know what tests were used to identify the tumor and what my other treatment options might have been. It would also have been good for the pathologist to receive credit for the work; my surgeon never mentioned pathology – I suppose he fancied himself as the star of the show!

Thanks to the pathologist's speedy diagnosis and the quick action the surgeon was able to take as a result, I can now look back on a potentially life-threatening situation as nothing more than a blip on my personal radar. I only wish all patients were as familiar with the contributions pathology makes to their care. Hopefully, as pathologists begin interacting more frequently with patients and doctors in the clinic, they will be.

For more patient-eye views of pathology, read our December cover feature, "Hello, My Name Is…"

Has Tumor Profiling Caught Up to Cancer?

Too many lung cancer patients experience needless treatment delays because their genetic data arrives too late. ddPCR-based liquid biopsy could be the solution.

By Scott Skibo, Pulmonologist at Haywood Regional Medical Center, A Duke LifePoint Hospital, Clyde, USA

Non-small cell lung cancer (NSCLC) is one of the most aggressive and deadliest forms of cancer. For treatment to be effective, especially in the latter stages of the disease, oncologists must take a personalized, genetics-based approach, traditionally informed by tissue biopsy. But these procedures are invasive and can take up to four weeks to deliver results (1). For optimal treatment, speed is of the essence. In my opinion, the best way to realize the true potential of personalized therapy is with a blood-based liquid biopsy testing strategy that uses a droplet digital PCR (ddPCR)-based assay.

In 30 percent of lung cancer cases, oncologists cannot use genetic information from a tissue biopsy to create a personalized treatment regimen (2,3). Some patients are ineligible for biopsies because the procedure is too risky; others simply yield too little tissue for analysis. And because the procedure can be highly invasive, physicians are often reluctant to perform multiple biopsies in a single patient – making it nearly impossible to monitor a tumor's shifting genetic profile via traditional tissue-based testing.

ddPCR-based liquid biopsies, on the other hand, only require a minimally invasive blood draw, but can detect genomic biomarkers in blood-borne tumor DNA shed by dying cancer cells. Many patients who are ineligible for tissue biopsy can still undergo liquid biopsies, and blood can be drawn multiple times with little harm, which means physicians can more easily and comprehensively monitor their patients' tumors over time.

In the clinic, ddPCR-based liquid biopsies also yield genetic data much faster than other commercially available tissue biopsy tests, which are primarily based on methods such as next-generation sequencing (NGS) (4,5). Although NGS has thus far been considered the gold standard for profiling tumors' genetic makeup, the quality comes with a downside: it yields so much data that pathologists may need up to three weeks to sort through it and deliver results (6). Consequently, 80 percent of the time, genetic data is

"Many patients who are ineligible for tissue biopsy can still undergo liquid biopsies."

not available to NSCLC patients by their first oncology consultation, and so a general chemotherapy regimen is started (7). And the delay in receiving more personalized treatment results in a worse patient outcome (4).

How do ddPCR-based tests deliver faster results? Unlike NGS-based liquid biopsies, they probe genetic hotspots directly linked to NSCLC, thereby providing only the information that immediately impacts a patient's treatment. To test the speed of a ddPCR-based liquid biopsy, a working group of five cancer centers offered a liquid biopsy for patients with NSCLC to their physicians – and 95 percent received results within 72 hours (4,8). With these turnaround times, physicians will almost always have patient data available in time to prescribe a personalized treatment regimen at the first visit.

"I see ddPCR as the future of personalized cancer therapies."

Moreover, ddPCR-based liquid biopsies are more cost-effective than traditional lung biopsies (1). Navigational bronchoscopies can cost over US$8,200; CT-guided lung biopsies run about $4,000 (and, in the 15 percent of cases where complications arise, costs can soar to over $18,000). Liquid biopsies, on the other hand, do not involve surgical procedures, so any phlebotomist can procure samples, reducing costs to an average of about $800.

I see ddPCR as the future of personalized cancer therapies. Based on our cancer center's successful experience with the technology, I believe it is only a matter of time before these kinds of blood-based liquid biopsies become an indispensable method for doctors to categorize a patient's disease and determine the best course of treatment.

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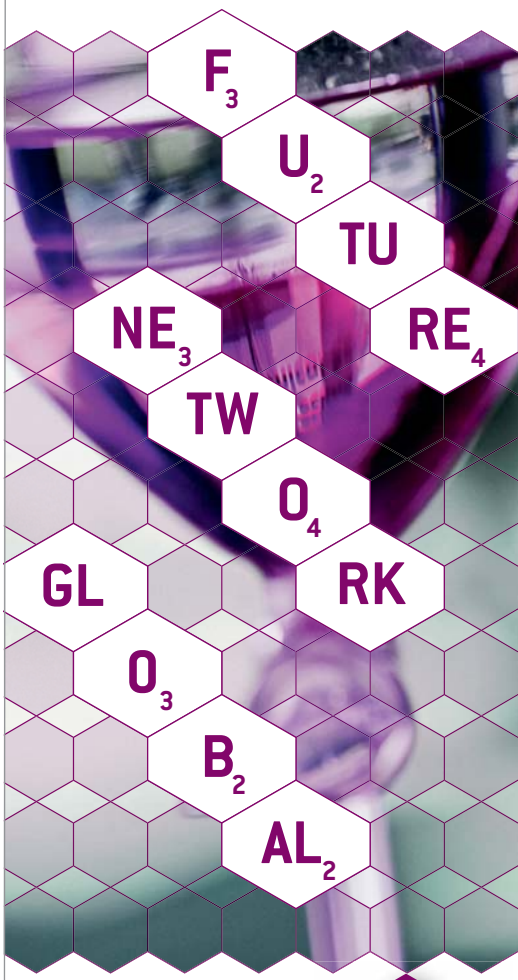

Highlights in 2018: Live Labs and Digital Transformation

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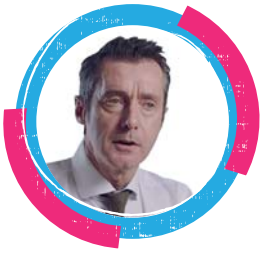
THE COMPUTATIONAL DISCUSSION CONTINUES...

In the first of this two-part feature (1), we discussed the impact and evolution of computational pathology. Now we ask: what can computational pathology do for you in the clinic? Many pathologists aren't sure – and some fear that computers may impact (or even take away) their jobs. But those working to develop artificial intelligence technologies for pathology see it as an assistive tool, not a replacement.

Our computational consultants



Manuel Salto-Tellez is Chair of Molecular Pathology at Queen's University Belfast, Clinical Consultant Pathologist at the Belfast Health and Social Care Trust, and Deputy Director of the Centre for Cancer Research and Cell Biology. A histopathologist and molecular diagnostician, he has studied and practiced internationally for the past 25 years. His current focus is on the integration of genotype and phenotype data for the improvement of personalized medicine and patient care.

Peter Hamilton is Business Lead for Philips Digital Pathology in Belfast, United Kingdom. Previously, he was Professor of Pathology Imaging and Informatics in the Centre for Cancer Research and Cell Biology at Queen's University Belfast. His research interests include digital pathology, computer vision, tissue bioimaging, and the high-throughput analysis of novel tissue and cell biomarkers.

How can artificial intelligence and machine learning contribute to the laboratory?

Manuel Salto-Tellez: From the point of view of discovery and application in pathology, concepts such as tumor microenvironment, cancer ecology and morphomolecular heterogeneity are beginning to reveal to us just how much information in tissues and cells remains untapped because it cannot be seen by the human eye. We need to understand the diagnostic and clinical relevance of this information. New information frameworks, such as the ones provided by AI and machine learning, will be critical to the further exploration of these concepts, and to helping us understand how we can diagnose and treat diseases faster and better.

Peter Hamilton: Artificial intelligence (AI) and machine learning will provide a way to automate the analysis of the complex tissue and cellular images that pathologists look at every day. Philips has been developing advanced algorithms that can be embedded within our digital workflow to analyze the pixels in digital pathology images and use the data to identify a range of abnormalities. From a provider's perspective, this has four major benefits for a digital pathology laboratory:

1. It can provide the pathologist with objective, reproducible data on the sample and thereby reduce diagnostic or prognostic variation, ensuring that clinical decisions are accurate and patients get the best care.
2. By implementing automated image analysis, some tasks can be accelerated, streamlining pathology workflows, removing inefficiencies and reducing costs.
3. Some diagnostic tasks may only be possible through AI; subtle changes in tissues, invisible to the naked eye, can only be detected using computer algorithms.
4. AI and machine learning can help integrate ever-increasing amounts of patient data – including pathology data – to establish individualized patient signatures and help drive precision medicine, targeted therapies and improved clinical outcomes.

What are the major pros and cons of AI in the clinic right now?

MS-T: The pros and the cons are both related to the volume of information. The amount of new data we can generate on a patient from multiple sources (genomics, pathology imaging, radiology imaging, clinical records, and even health information on social media) is phenomenal. Equally intimidating are the challenges involved in identifying the right algorithms to translate such a wealth of information into practical considerations. This translation arguably represents the most important hurdle to applicability. Crucially, AI and computational learning will help us analyze this massive amount of information to make it meaningful. With improved understanding, we can respond by developing solutions – and, hopefully, cures.

PH: AI-based algorithms need sufficient robustness to be able to handle the variation in sample preparation that exists between different laboratories. And that requires the use of extensive datasets to train and validate algorithms for clinical pathology. My colleagues and I are working with multiple partners, including pathology labs that have already moved to 100 percent digital workflows, to build some of the largest libraries of digital images across multiple disease types. This growing digital library of annotated images will help build robust AI for all of our future applications.

As Manuel says, the ability to integrate multiple sources of data is indeed a critical driver for AI in the lab. Pathologists are skilled integrators of complex information. AI can provide important tools that enable pathologists to do this more effectively across multiple data sources and multiple patients. The data management question definitely extends beyond pathology into other specialties where the level of available data – and the complexity of that data – is increasing year on year.

In one research study on the identification of lymph node metastases in breast cancer (2), the pathologist slightly outperformed the AI algorithm in correctly identifying metastases. However, when both the pathologist and AI analyses were combined, the overall accuracy rate significantly increased over what the pathologist was capable of alone. This example shows the real value of combining the skills of the pathologist with AI to reach a more precise diagnostic assessment of a case. I think it's clear that hybrid machine/human intelligence has the power to drive major improvements in pathology in many ways.

Underpinning all of this is patient safety. Clinical and regulatory clearance for AI tools is of the utmost importance and is a very costly part of the process – probably the most expensive step. We have to perform the most robust clinical studies on our pathology AI tools and obtain full regulatory clearance before releasing them onto our digital pathology platform. That way, we ensure that pathologists can apply new tools with real confidence.

Is AI going to replace pathologists?

PH: To be clear, AI is not going to replace pathologists; rather, it's going to enrich workflows and help pathologists better cope with the rapid rise in sample numbers and their complexity. We are currently working with large teams of pathologists to build exciting new tools – and many pathologists cannot wait to get their hands on them!

MS-T: Peter is absolutely right. Like many other past and present developments in pathology, AI is not here to substitute for pathologists, but to improve the quality of our diagnostic output. It is conceivable that the pathologist of the future will be involved in not only diagnostic interpretation, but also in the application of algorithms to images – and that the final diagnostic opinion may be a combination of both.

Can you describe an "ideal" situation for the application of AI and machine learning?

MS-T: In my opinion, digital pathology and AI can potentially be of help in five main areas of the "pathology diagnostic pathway."

1. Finding a new way of managing images in routine diagnostics and in routine archival collections;
2. Integrating images with existing patient and pathological information more readably (LIMS, image reference resources, can texting, access to diagnostic bibliographical resources, and so on);
3. H&E interpretation and resolving areas of diagnostic dilemma (for instance, in situ versus invasive cancer);
4. Biomarker scoring, immunohistochemistry and in situ hybridization; and
5. Annotating samples ahead of nucleic acid extractions and molecular testing.

Thus, the ideal situation is "global" and, in many ways, limitless!

PH: There are many areas where AI can be used to support diagnostic reporting and reduce inefficiencies in pathology workflow. For example, it has been shown that deep learning AI can be used to rapidly detect lymph node metastasis in breast cancer patients with high levels of precision. Grading certain cancers using conventional diagnostic schemes and the human eye is also known to be associated with subjectivity and error. Computer-based AI and image analytics will certainly help ensure higher levels of consistency and precision across pathologists and better management for patients. In laboratory workflows, I can also envisage ways in which AI can intelligently manage workloads, dispatching cases to distributed pathology teams in ways that drive efficiencies, reduce costs and accelerate turnaround times. Such advances will be critical in overburdened pathology laboratories across the world.

What would you like pathologists and lab medicine professionals to know about AI in the laboratory?

PH: I would say two things. First, AI – as with any new technology in pathology – will really help enrich your ability to practice pathology and improve your ability to serve your patients. Second, AI is not here to remove pathologists from the decision-making process. It can and will help accelerate certain steps in pathology, but pathologists will continue to be responsible for the final decision. Pathology will change with the introduction of AI in the same way that it changed with the introduction of IHC, ISH, molecular pathology and now next generation sequencing – but, as with these advances, it will provide richer data on the interpretation, understanding and integration of morphology. I see this as an exciting step forward in the discipline of pathology – one that puts pathologists at the very center of clinical care and the delivery of precision therapeutics.

What kinds of equipment, software and training are required to implement clinical AI?

PH: To begin with, AI requires the implementation of digital pathology in routine diagnostic practice – without this, AI applications in pathology are not possible. As we see the adoption of digital pathology for primary diagnostics grow across the globe, AI is following close on its heels. Indeed, in many laboratories, AI and computational pathology are becoming key drivers for the adoption of digital pathology due to the efficiencies they can bring.

In addition, for AI to be truly effective, it needs to be seamlessly embedded within the pathology workflow. At Philips, we are building the technologies that will allow image AI to be an integral part of our existing digital pathology solution and diagnostic workflow experience. The goal? To make AI simple and accessible to pathologists, with results available at the time of review. We want to provide powerful decision support tools for pathologists to help them make the toughest clinical and therapeutic calls.

As with any new technology, pathologists will have to be trained

in digital and computational pathology. It's important to make that digital transition as straightforward as possible, providing technologies that enable digital pathology workflows rather than making them more complex. These include high-throughput scanners that have "walk away" technology and don't require multiple technicians to run the instrumentation, image management and storage that is easily scalable, and user interfaces that have been designed by pathologists to enrich digital workflows and accelerate AI-based decision-making. Approaches like these reduce the level of training and support that pathologists will need to adopt digital pathology. The same applies to AI; the tools need to be easy to apply and provide results that can be easily interpreted and embedded into the diagnostic report. Having an existing, highly streamlined digital pathology workflow allows Philips to incorporate AI in a way that saves pathologists effort, something I hope will become standard practice as digital pathology becomes the norm. Pathologists won't need to be data scientists or computer engineers to use AI; it will become a normal part of their diagnostic decision-making.

What advice do you have for pathologists moving to a more computational environment?

MS-T: Take the example of molecular diagnostics. Many tissue pathologists arrived late to this important area of diagnostics, and have thereby put it in the hands of others. Equally, not embracing AI from the beginning and allowing others to enter the field on our behalf may be devastating for the future of our discipline. If we successfully adopt AI ourselves, though, we will have engaged in a new diagnostic revolution – not dissimilar to the one Virchow led more than a century ago.

Every time new "glass" pathologists are involved in studies in which they become "digital" pathologists, they typically go through five phases: reluctant, skeptical, interested, involved, and enthusiastic. The "knowledge integration" that is facilitated by working in silico is an unequivocal advantage to almost every pathologist who gives digital pathology a fair try.

PH: I would advise pathologists and laboratory medicine professionals to work closely with industry to embrace change, and to ensure the reliable and effective implementation of new technologies in a way that truly enables best practices, processes, and workflows for your laboratory. It is equally incumbent on industrial vendors to truly partner with pathologists and laboratories to ensure successful digital transformation and the effective introduction of new AI technologies.

The only other advice I would give pathologists during this period of transition is to continue to be excited by pathology as a discipline and the future advances that are within our reach! Your role in defining that future is critical to pathology and to the patients you serve every day.

What's happening right now with AI implementation in pathology?

PH: Philips is committed to developing AI applications for digital pathology users. We have also responded to an opportunity to work with the UK's National Health Service (NHS), government, research organizations, other industries and the small and medium enterprise community to explore how we can apply AI to pathology to advance patient care in a fully digitized laboratory. In particular, we are looking at what we can offer to support the incubation and creation of world-class digital pathology hubs within the NHS, and to drive leadership in the transformation of diagnostic pathology. At the moment, we are working with the Office of Life Sciences and the Department for Business, Energy and Industrial Strategy to determine how best we can collaborate to drive improvements in NHS pathology and in AI research within the UK.

What's next for computational pathology?

PH: Health knows no bounds. Philips conducted a recent global survey and found that 56 percent of people who had had a cancer-related experience felt that "connected care" technology made their experience more positive (3). We see digital pathology and AI as being cornerstones to connected care; they are keys to improving patient care and health outcomes. The digital transformation is happening – and will continue to happen – in medicine and in pathology. It's nothing to be scared of, and it will be part of a broader digital revolution in health. It's a genuinely exciting future – one that pathology and pathologists deserve.

MS-T: For the last 60 years, we have witnessed the "genomic revolution in medicine," and we are now beginning to see tangible gains. I believe we are beginning to experience the "AI revolution in medicine," a concept that encompasses genomic information and other sources of information – one that will provide a true "integromics" approach to healthcare.

To read Part 1 of this feature, please see our January issue.

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A STRATEGY FOR SUCCESS

How the UK's Life Sciences Industrial Strategy can take digital and computational pathology to the next level

By Chris Hudson

The Life Sciences Industrial Strategy is the UK government's attempt to identify areas of potentially sustainable change in healthcare and research. It came about as a result of the country's vote to leave the European Union. In the course of evaluating the assets that we as a country possess, the National Health Service (NHS) realized that life science is a £64 billion industry here – a jewel in the UK's crown. Academia and industry are huge; we have three of the top 10 universities in the world and a hotbed of spinout companies. I think, as a country, we have a great deal to offer – and I think digital and computational pathology will be an ongoing strength. The UK is a well-respected country in terms of its academic and research capabilities. In many countries, digital and computational pathology still have low adoption rates. We now have the opportunity to develop those technologies, use them to take our pathology service to the next level, and then even market them. If we start developing and validating pathology algorithms, we can then not only improve our own healthcare services, but also potentially help other countries improve theirs.

Building Rome

Change is not going to happen overnight. The fact that the government is now making digitization a priority is already a huge step; that means they will start to look at the funding and organization that will be necessary to roll out a fully digital pathology service. At the moment, I understand that they are looking at piloting a program across five to six hubs before a complete rollout. It will be important to select the right hubs – ones that are already Centers of Excellence in digital pathology – so that they can try to implement at pace and scale. To pathologists and laboratory medicine professionals, I would say, "Watch this space." I think things will become much clearer in the next three to six months – more announcements, more ways to get involved… This is just the start!

We have opportunities to completely change clinical practice – and the driving forces to make it happen, too. Pathology is what we call a "greying profession," with a lot of pathologists due to retire in the next few years and vacancy rates of up to 25 percent. But with a growing, aging population and an increasing disease burden, the role of the pathologist will only become more important over time. The Royal College of Pathologists estimates that around 20 million slides are examined each year in the UK (1) and that the increasing demand for pathology services as indicated by the Keele Pathology Laboratory Benchmarking Programme exceeds the growth in the number of consultant pathologists each year (2).

The most valuable resource in any pathology lab is the pathologist – so we need to use them and their skills, knowledge and experience in whatever way is most beneficial for patient outcomes and service sustainability. And, in my opinion, that means the growing adoption of computational pathology practices.

Digital pathology technology (for instance, in terms of scanning) has improved greatly over the last decade. I think we are now at the stage radiology achieved about 15 years ago when picture archiving and communication systems (PACS) were widely introduced. Those machines revolutionized the way that care was delivered, particularly with respect to turnaround times, and I think digital and computational pathology will do the same. These tools enable pathologists to use their skills in a wiser and more targeted way. PACS didn't happen overnight; it was rolled out over a period of time, with test cases prior to full implementation. These "digital exemplars" will help the NHS establish best practices and tackle the practical realities of digitization.

If you couple the move to digital with artificial intelligence (AI) and machine learning, pathologists will be able to focus their efforts on the cases that really require their time and attention – not on a barrage of ordinary negative results. If we can have AI tools screen out cases that don't require a pathologist's eye, we should see faster diagnoses, more efficient use of pathology resources, and closer attention to more complex cases that need specialist care. I think the rise of computational pathology will really take the field into the future and ensure that we have sustainable pathology services for as long as we need them. Ideally, pathologists will get involved as soon as possible – not only will it help them to keep up with the changes, but it will enrich their working lives, ease their strain, and improve patient care, too.

Are our jobs safe?

If you look at the raft of companion diagnostics coming down the road, differential diagnosis is becoming more difficult. Some say that,

as our molecular abilities improve, we no longer need histology – but I don't believe that at all. We need every aspect of pathology as much as ever, and that means we need pathologists. We need not just data, but interpretation as well. Technology is not the answer to all of our problems; it's a tool that enables pathologists to do their job in the best possible way.

There are already a number of algorithms that can help with diagnosis and treatment decision-making; we simply need to ensure that we can adopt them. Technology advances so fast that sometimes ethics and regulations lag behind – but we need to catch up in that respect. Algorithms are the tools that will free up pathologists' time to prioritize appropriately. They are the aids that will help decide which slides need to be referred to specialists. Obviously, this will take some time; we have to not only develop and refine these algorithms, but also train and validate them. The more an algorithm is used, the better the machine becomes at implementing it. They are not always right, but the more data they acquire, the more precise they become. It's no different to a shopping algorithm that suggests products based on your previous purchases and browsing habits!

A blue-sky future

The ideal digital pathology laboratory of the future would be holistic; it would bring together all of the pieces of information – histopathology, molecular pathology, sequencing – for pathologists. With a complete picture of the patient, they'll be able to take a much bigger role on the multidisciplinary team, and in patient care in general. They become the guardians of advice and knowledge.

What else do I hope we'll have in our blue-sky future labs? I'd like the ability to see and link databases. The pathologist could examine the patient's histological, molecular and genomic status, then search for similar patients and see what courses of treatment have been most effective. The possibilities are endless – and that's the great thing. I think pathology is going through a very difficult time at the moment; there are more and more demands being put upon pathologists. But I believe that, if we embrace new digital and computational technologies, we have a bright and rosy future ahead. If technology can free pathologists to do the things that really matter, how great is that?

Chris Hudson is Director of Healthcare Development and Strategic Services at Roche Diagnostics, Burgess Hill, United Kingdom.

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RISE OF THE COMPUTERS

Are diagnostic algorithms ready to take their place alongside pathologists?

By Babak Bejnordi

It's true that some pathologists fear the loss of their jobs to computer algorithms and other artificial intelligence (AI) technologies. But how reasonable is that fear? How likely is it that diagnostic algorithms will be able to perform the tasks that, up until now, have required the eye and intuition of a human pathologist? And what, ultimately, will be the role of the machine in the pathology laboratory of the future? The first step to implementing AI in the pathology workflow is to deploy digital pathology, which involves scanning glass pathology slides and storing the resulting images in a digital repository to be viewed on computer screens. As soon as a digital image has been acquired, AI algorithms can analyze the data and provide additional information. The pathologist can have the results ready as soon as they start reviewing the images, meaning that they can use that additional information data to provide a deeper, more nuanced diagnosis.

Advances in slide scanning technology and cost reduction in digital storage capacity are now enabling full digitalization of the microscopic evaluation of stained tissue sections. One of the most significant benefits of these technological advancements is the possibility for computer-aided or computerized diagnosis – not for every patient or every disease, but in a way that permits pathologists to focus their attention on the cases that most need it. AI techniques enable computers to solve perceptual problems, such as image recognition, which could lead to drastic improvements in pathology diagnostics in terms of objectivity, accuracy, and efficiency. In my opinion, AI is not a replacement, but an excellent support system for pathologists.

The CAMELYON16 challenge

My colleagues and I organized the CAMELYON16 challenge (CAncer MEtastases in LYmph nOdes challeNge) to evaluate state-of-the-art machine learning methods for the detection of metastases in sentinel lymph node tissue sections – and to compare their performance with trained pathologists. The majority of the algorithms submitted for the challenge were based on deep artificial neural networks, which are at the forefront of machine learning algorithms. Deep neural networks consist of multiple layers of interconnected artificial neurons; these algorithms seek to draw a relationship between an input (for instance, a diagnostic image) to an output ("cancer" or "benign"). To build the system, we expose the deep learning algorithm to a large dataset of labeled images, with which it teaches itself to identify relevant objects. During the learning process, connections in the deep neural network become stronger or weaker as needed to make the system better at prediction. After training, we can apply the network to images it has never "seen" before.

CAMELYON16 was the first grand challenge in pathology to offer a large collection of whole slide images (a total of 700 GB of image data!), helping researchers around the world bridge the gap from working on small region-of-interest images to whole slide scans. In this challenge, every

"There are already a number of algorithms that can help with diagnosis and treatment decision-making; we simply need to ensure that we can adopt them."

"Pathologists may spend less time on the interpretation of pathology slides, and instead focus on more critically important tasks."

participant (whether digital algorithm or human pathologist) was given one single slide per patient from which to determine the presence or absence of breast cancer metastases. In a real clinical setting, however, we would evaluate sections from multiple levels. The test dataset was also enriched with cases containing metastases. The majority of specimens pathologists encounter in daily practice do not contain metastases – so this enrichment was necessary to achieve a well-rounded representation of what might be encountered in clinical practice without including an exorbitant number of slides or several terabytes of total data. At the moment, the majority of pathologists worldwide interpret pathology specimens under a microscope. The reason challenges like CAMELYON16 – and now CAMELYON17 – exist is because the next generation of pathologists will be working with digital images day in and day out. We'll need appropriate algorithms to help them manage the sheer volume of data they will encounter, and we'll also need to ensure that these pathologists are trained to use AI and computational methods in their daily practice.

A new assistant

The results of our article on the diagnostic assessment of deep learning algorithms for metastasis detection (1) showed that there is a significant opportunity for AI in pathology – namely, assisting pathologists with the interpretation of histopathologic sections. I suspect that, as technologies advance and adoption increases, we will see more applications of AI in pathology in diagnosis, treatment, outcome prediction and prognosis evaluation.

AI is increasingly being recognized as a major element of the overall healthcare landscape. We are now at a turning point where computers perform better than clinicians at specific tasks. This offers a great opportunity to empower clinicians by improving their efficiency and accuracy. That's not to say that I think AI will replace clinicians; rather, it will gradually change the way they work. Pathologists may, for example, spend less time on the interpretation of pathology slides, and instead focus on more critically important tasks, such as aggregating data from multiple sources (molecular, genetic, radiological, and so on) to better understand patterns that lead to more accurate and definitive personalized diagnosis. Rather that neglecting AI or feeling threatened by it, I think medical professionals – and pathologists, in particular – should embrace AI solutions. There's a bright future ahead for human-computer collaboration!

Reference

1. *B Ehteshami Bejnordi et al., "Diagnostic assessment of deep learning algorithms for detection of lymph node metastases in women with breast cancer", JAMA, 318, 2199–2210 (2017). PMID: 29234806.*

Babak Bejnordi is a senior deep learning and computer vision engineer working on development of intelligent vision systems and AI for autonomous driving. Previously, he worked on the application of machine learning techniques to the analysis of histopathological images at Radboud University, the Netherlands, where he obtained his PhD.

AN EYE ON AI

Algorithms that help screen for eye diseases can make the overworked diagnostician's life easier

Histopathology is not the only diagnostic arena that could benefit from computer support. In ophthalmology, for example, many instances of eye diseases go undiagnosed because of a lack of trained professionals to handle the vast amount of necessary screening. Algorithms capable of distinguishing referable images from those that don't require extensive human scrutiny can help lighten the load – and could potentially be the answer to the challenge of overworked, under-resourced diagnostic specialists. Here, we gain the perspective of Daniel Ting, Assistant Professor at the Singapore National Eye Center, SingHealth Duke-NUS Medical School, National University of Singapore.

What developments have led to the rise of artificial intelligence in medicine?

AI has been around over the last few decades. Over the past few years, deep learning using graphic processing unit (GPU) servers has revolutionized the field of computer science, sparking tremendous interest in image recognition, speech recognition, and natural language processing. In medical imaging, deep learning has shown to be comparable, if not superior, to human graders in detection of many medical conditions, including diabetic retinopathy, skin melanoma, breast metastases and tuberculosis.

Tell us about the deep learning system you developed for retinal disease screening...

Our system is effective in using retinal images to automatically detect three conditions: referable diabetic retinopathy, glaucoma, and age-related macular degeneration. We are

"AI as a diagnostic tool has several obvious advantages, including cost and time savings, a sustainable workforce that will get smarter over time, and zero intra-rater variability."

currently in the midst of developing algorithms for other retinal conditions, including retinal vein occlusions and retinal detachment.

The designs and concepts of AI algorithms are fairly similar across different medical disciplines. Most importantly, we need to know the right research questions to ask so that we can design the algorithm accordingly. Training datasets with labeled "ground truth" – that is, known results the computer can learn from – is equally (if not more) critical than the technical architecture of a deep learning system both within and outside ophthalmology. For instance, I am involved in several AI grants in radiology at Johns Hopkins University, looking at developing a chest radiograph algorithm to differentiate normal from abnormal X-rays and identify different lung diseases. Eventually, I hope to also apply AI to dermatology and tissue pathology.

What are the pros and cons of using AI as a diagnostic tool?

AI as a diagnostic tool has several obvious advantages, including cost and time savings, a sustainable workforce that will get smarter over time, and zero intra-rater variability. Our system in particular can reduce the total diagnostic workload by at least 50 to 70 percent, simply by removing the non-referable images so that the human graders can focus on the retinal images that need extra attention. AI is also useful for lifelong monitoring; once the machine has baseline patient data, it can easily compare future images to signal the progression – or resolution – of disease.

That's not to say that there are no disadvantages to AI in the clinic. It requires a large dataset to train, for instance, which is not only time-consuming, but also creates a need for technical expertise and supporting infrastructure. The challenges are easing every day, as cloud-based services become cheaper and more readily available, but effort and expense will remain. In my opinion, the benefits of AI are certainly worth the process of establishing, training and maintaining it!

What lies ahead for your work – and for medical AI in general?

Our team has come a long way. My four co-inventors (Tien Wong, Wynne Hsu, Mong Li Lee, and Gilbert Lim) and I began developing and testing this AI system five years ago using retinal images we collected over a decade ago. We've poured enormous manpower and financial resources into this project, and I'm thrilled that the system has overcome all of the initial obstacles and now shows promise in assisting diagnostic professionals (1).

AI is the fourth industrial revolution in human history. As far as I can see, it's definitely set to revolutionize medicine over the next few decades. In ophthalmology, we hope that AI will help with the repetitive diagnostic workloads for diseases like diabetic retinopathy, glaucoma, and age-related macular degeneration. Using different retinal imaging modalities, AI can potentially help us see the changes to retinal neurovascular structures that have thus far been invisible to the human eye, and help us predict the incidence and progression of various retinal conditions and even systemic diseases. By having a robust algorithm, we also hope to deliver personalized medicine to the global population of patients with diabetes. There are similar trends in other medical specialties – not just ophthalmology and pathology, but also radiology, dermatology, oncology, and others.

Reference

1. *DSW Ting et al., "Development and validation of a deep learning system for diabetic retinopathy and related eye diseases using retinal images from multiethnic populations with diabetes", JAMA, 318, 2211–2223 (2017). PMID: 29234807.*

TO ADOPT AND INTEGRATE

Artificial intelligence has a lot to offer pathology – but we need to make sure we're keeping up

By Jeffrey Golden

The recent and rapid adoption of AI in healthcare reflects the coalescence of multiple developments – including computer hardware advances that permit the rapid and effective processing of large datasets, such as those derived from pathology images. In particular, I think the field has been pushed to new limits by improvements in pathology imaging technologies and the recent application of convolutional neural networks and other methodologies to medical problems.

There are good and bad aspects to the changes that AI will bring. The advantages of using AI to interpret slides include increased efficiency, improved diagnostics and better patient stratification, as well as the ability to perform intermediate patient response assessments with greater accuracy. With computers doing the routine tasks at which they excel, pathologists will be free to focus on difficult and unresolved issues. My concern, though, is the potential for over-application of AI without sufficient oversight and interpretation by a pathologist. Many of my colleagues worry that computers will take away their jobs. I don't worry about that – I worry that they'll allow it!

The other comment I often hear is that pathology's transition to digital mirrors radiology's over the past decades. That is not entirely the case; there are several key differences, but the main one is costs. For radiology, there were clear financial advantages to adopting digital images. Those same advantages do not exist in pathology; for us, the move leads to added costs, which will demand defining a value proposition. I believe that value exists, but it is incumbent upon us to prove it to funders and governing bodies. There is also a data storage (and cost) issue. The data files for pathology images are significantly larger than those of radiology. As a result, the cost of storing the data and moving it around is significantly higher.

The one issue that has not been well-addressed is training. If we want to fully integrate computational pathology into the laboratory workflow, we need to be training this generation of pathologists – and the next – to do that. It's not yet happening at any scale. Adoption of didactic as well as practical education in digital pathology and computational methodology will be essential for all training programs to adequately prepare pathologists for future changes in healthcare delivery.

What does the future of computational pathology look like? In the near term, I expect an explosion of publications in the realm of AI. This will be paralleled by greater implementation of digital imaging in pathology for routine diagnoses and second opinions – the latter an area where it is already commonly used. Both elements are required for the broad adoption and integration of AI. After that, my best guess is that we will soon see the adoption of diagnostic stratification of neoplasms with regard to prognosis and treatment based on deep learning algorithms – and from there, who knows?

Jeffrey Golden is Chair of the Department of Pathology at Brigham and Women's Hospital and Ramzi S. Cotran Professor of Pathology at Harvard Medical School, Boston, USA.

THE DEVIL'S ADVOCATE

Not every pathologist is a digital expert – and concepts like "computational pathology" can be intimidating to those who are not. So what does it really mean?

By Anil Parwani

"Regular" pathologists have a somewhat nebulous view of digital pathology – it's an emerging technology that may be able to help them with some aspects of their workflow. Many of them have not completely embraced digital pathology yet, though. They are waiting to see more widespread adoption and ease of regulatory burden – perhaps quite wisely. Computational pathology is an even more uncertain prospect; most people don't yet understand the term very well. Many pathologists do understand digital image analysis and artificial intelligence, but that doesn't necessarily mean that they are believers in the technology – at least, not yet.

Why would anyone feel hesitant about, or even threatened by, digital and computational pathology? In my opinion, the main reason is that many pathologists feel that computers are not ready to make complex diagnoses yet – which is certainly true; they're only equipped to provide assistance to the pathologist whose job it is (and whose job will remain) to make such diagnoses. Others feel that by supporting such technology, they put their own jobs at stake; why should they "train" the computers to take away their work? Still others worry that digitization may result in the outsourcing of their jobs to other countries, or to commercial labs with established computational pathology pipelines. Viewed from the perspective of the individual pathologist, especially one without the resources to remain fully up to date with new technologies, it's no surprise that computational pathology is intimidating!

I think, though, that the true advantages and disadvantages of these technologies are slightly different. Computational pathology's key benefit is that it offers an integrated view of the patient. The software can produce high-quality images that include annotations and clinical metadata, so that the pathologist can see the complete picture. Along with computer-based quantitative and qualitative image analysis, the overall practice of pathology becomes more objective – and thereby also more reproducible. And, of course, every pathologist is familiar with the tedium of counting and segmenting; why not assign these tasks to computers, so that we can do the more challenging, high-level diagnostic tasks?

I'm not worried that a computer will take my job. Why? Well, the kinds of computers that can provide practical diagnostic assistance are not cheap. They also require large numbers of annotated images to learn, so training them is no simple task. And many of the diagnoses we make in the clinical setting are too complex – or too dependent on morphology and molecular data – to entrust to even the most advanced computer.

My only real concern, in fact, is that humans may begin to rely on computers too much. We must take care not to become complacent and stop checking the computer's results properly. At the end of the day, the responsibility for the patient's diagnosis still falls at our feet, not the virtual feet of our software.

Often, those who develop or market new technologies feel compelled to tell us how good those technologies are, but that isn't always the right approach; hearing how computers can do

"My only real concern, in fact, is that humans may begin to rely on computers too much. We must take care not to become complacent."

everything a pathologist can do – or even better – is threatening for some. It's far more useful to learn how technology can help us in our daily workflow, how it can assist us in sharing and analyzing images, and ultimately improve the speed and quality of our diagnoses.

Those of us who regularly use computational pathology tools can help, too. We can talk about the practical benefits we've encountered. I, for example, particularly benefit from diagnostic algorithms, telepathology, and help with quantitating biomarkers and structures. Such tools have helped me to reduce error and facilitate a digital workflow. They also enable researchers and clinical teams – whether down the hall, in a different building, or on opposite sides of the globe – to share and review images instantly, then use algorithms to obtain valuable insights that ultimately lead to a more informed, more detailed diagnosis and personalized care plan for each patient. In my experience, combining pathologists and computers only results in better pathology with powerful tools for diagnosis and decision support.

Anil Parwani is Professor of Pathology, Vice Chair and Director of Anatomic Pathology at The Ohio State University and Director of Pathology Informatics and the Digital Pathology Shared Resource at The James Cancer Hospital, Columbus, USA.

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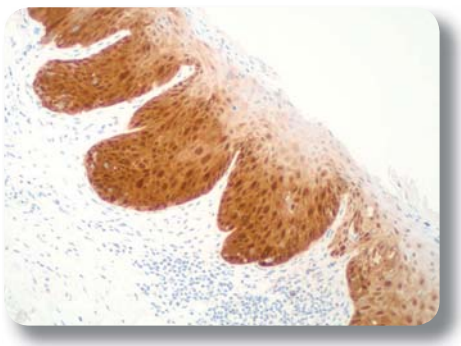

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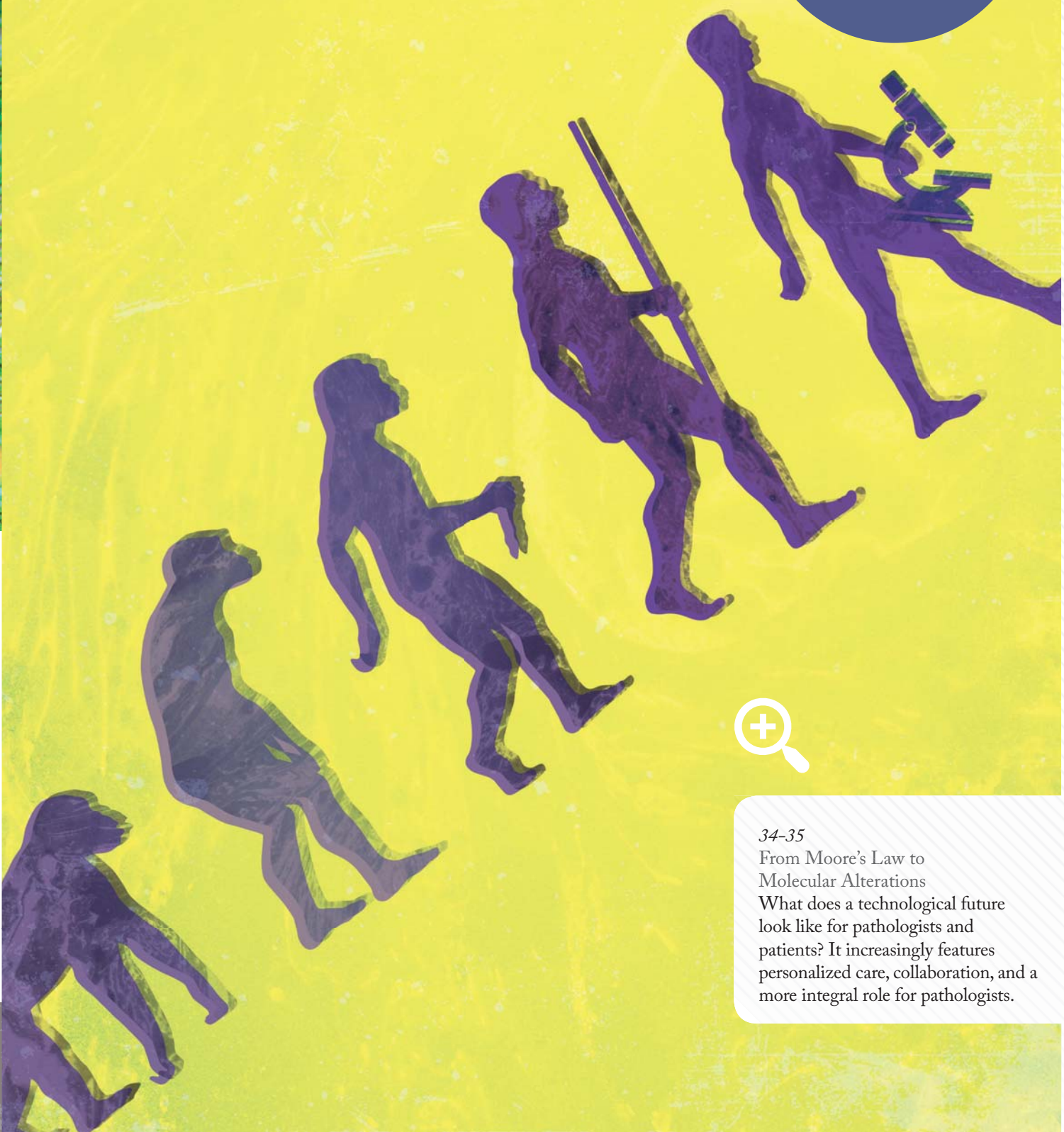
Workflow



From Moore's Law to Molecular Alterations

Pathology's future is personal – and pathologists are in prime position to guide the inexorable rise of technology

By Philippe Tanière and Matthew Evans

The landscape of patient care has undergone significant changes in recent years, particularly with regard to new diagnostic technologies. Highly effective treatments have become available for many difficult-to-treat diseases but, to benefit, patients must first be tested for specific, clinically relevant molecular alterations. The growing need for molecular testing drives the development and implementation of technologies that make the process as quick, efficient and accurate as possible.

After a tissue sample undergoes morphological and immunohistochemical assessment, the remaining material is used for molecular testing (the details of which vary depending on tumor type, tissue of origin, and available treatments). To make the best use of the available sample, we need to look at various different types of molecular alterations, which means that we need to use multiple different platforms.

At a Glance

- *Molecular testing is growing increasingly important in personalized care*
- *Collaboration between pathologists and other clinical teams is essential for the best patient outcomes*
- *New technologies will help pathologists to play an even more integral role in patient care*
- *The change is bigger than any individual pathologist – and we're going to have to work together to move the field forward*

Many targets, many platforms

Full profiling of a tumor requires assessment of multiple targets of different types and often involves looking at aspects of gene mutations, chromosomal alterations and protein expression. We can't achieve all of that by using just one technology – so we need to choose the combination that best meets our needs. The selection of technologies to assess these targets requires careful consideration to ensure that the techniques are timely, accurate, and suitable for (often extremely small) real-life samples. Turnaround time, cost and accreditation also need to be taken into account. The best example of this is lung cancer, in which there are now several targetable molecular alterations: *ALK* translocation (a chromosomal feature), *EGFR* mutation (a genomic feature) and *PD-L1* expression (a protein expression feature). All of these results are dependent on each other, so oncologists need all three at once to know how to treat a given patient – and it has to be done using a very tiny biopsy, with the results available very quickly, so that treatment can be initiated without delay.

The reality is that a laboratory needs to have multiple platforms accredited and ready to use. We need platforms for DNA and RNA assessment; in our lab, for instance, we have three real-time PCR platforms, pyrosequencing technology and next generation sequencing facilities, which are all used every day on both tissue and plasma to fulfill our service's daily requirements. We also use two types of automated immunostainers and fluorescence in situ hybridization technology to assess for protein and chromosomal alterations. The molecular profile demands integration of the information coming from all these platforms, performed in parallel. Consequently, the logistical implications are enormous – but the selection and preparation of samples is key. It's possible that multiplex assessment of both gene and chromosomal alterations on real-time PCR or NGS, and protein/chromosomal alterations using multiplex algorithms on digitized slides, could become standard in the future, but we feel that this will be a gradual process and that, for the foreseeable future, we will need to maintain a wide variety of equipment.

Plasma testing allows rapid, noninvasive assessment of particular alterations and should be regarded as complementary to tissue testing in certain clinical circumstances, rather than a full surrogate. It will not replace the morphological diagnosis of cancer and the assessment of protein expression, but could become increasingly useful in gene and chromosomal alterations as long as the clinical sensitivity is acceptable. (At the moment, because we can't distinguish tumor from non-tumor circulating DNA, the lack of detection of a targetable alteration on plasma cannot be regarded as a final result.) The possibility

of plasma testing is huge in the context of monitoring patients to assess treatment efficacy and to detect early recurrence; however, clinical studies are needed for every marker in every type of tumor to validate them in clinical contexts.

With these new technologies, I have great hope that we will be able to further improve patient care. By looking for variants beyond those currently known to be clinically relevant, we could potentially identify new markers for more accurate prognoses and better predictions of treatment response.

Slide scanning is an example of a technology that – while already making a difference – is still developing. Nonetheless, it opens up the possibility of multiplex assessment of protein expression (rather than just gene alterations), which will likely become increasingly important in the future, especially in selecting patients for immunotherapy. Paired with sequencing, which has become a routine process in clinical laboratories, these complementary technologies allow us to deliver optimal treatment. As the healthcare landscape continues to evolve, technological advances will continue to make it easier for us pathologists to do the best for our patients. However, there is one aspect that's more valuable than technology when it comes to ensuring optimum outcomes.

Collaboration is key

It's well-established that collaboration between multiple clinical teams (including pathologists, surgeons, oncologists, and radiologists) is essential for optimal patient care. The pathologist's work increasingly involves integrating complex information from multiple sources – morphology to protein expression to molecular alterations. Integration of this information, particularly in the context of molecular changes, demands extremely close work with laboratory scientists. Likewise, the synthesis of this information with clinical and radiological data demands that we work very closely with clinicians. It's a group effort that extends beyond laboratory or clinic work and into multidisciplinary meetings.

The case of unknown primary carcinoma is a good example of the effectiveness of such cooperation. Recently, a patient presented to physicians with a mass in the liver. A biopsy showed adenocarcinoma, but immunohistochemistry gave no specific clues about the origin. The discussion at a multidisciplinary meeting revealed that there was also a lung lesion, which could not be biopsied. The team decided that the liver biopsy should be assessed for molecular alterations that might link to lung cancer (for instance, *EGFR* mutations, *ALK* translocations, or *PD-L1* expression). Ultimately, the team identified a mutation conferring sensitivity to anti-*EGFR* drugs, and the patient was able to commence treatment for presumed metastatic lung cancer.

It's worth pointing out that discovering an *EGFR* mutation in a malignant tumor does not necessarily convey any useful information on its own. The alteration only has predictive use if it is identified in lung cancer. That's because the value of any specific alteration in a particular type of tumor must first be demonstrated in clinical trials. No mutation is agnostic for tumor type because of the enormous and poorly understood complexity of signaling pathways in cancer cells. It's also worth mentioning that the presence of an actionable mutation is never a guarantee of response to a particular drug; it conveys only a statistically significant increase in likelihood of response.

Managing a technology-driven future

As pathologists, we do not regularly see patients ourselves – but by attending multidisciplinary meetings, we receive regular feedback about patients we've tested. We're also increasingly seeing those patients return for secondary resistance mutations months or years later – so, in a sense, that's a kind of feedback, too. Systematic auditing and data collection is essential in monitoring these patients, and that data is always worth publishing to keep the community informed.

Pathologists sit at the interface between clinical practice and testing technologies. As technology develops and there's a tendency to investigate as much as possible, I think the pathologist's role will become increasingly focused on utilization management – using their clinical knowledge to limit testing to what is actually going to inform clinicians and help patients. All in all, pathologists are applying their medical background – in tandem with their technical knowledge – to make a real impact on patient care in a way that has never been done before.

As the future becomes more technology-driven, I don't think molecular testing will not take place in all pathology departments; instead, I believe it is likely to be limited to large, centralized hubs. However, the advent of digital technologies allows every pathologist to access all of the clinical and molecular data required to personalize patient care – even from distant locations – and, as a result, pathologists working today and in the future will need to move to a more patient-centric approach. No individual pathologist can lead this move, because no one person can have all of the answers. A change this big requires the involvement of the profession as a whole; everyone must get on board.

Newly available technologies open up more opportunities and create the potential for the field to grow – but, if we are to achieve the ultimate goal of personalized medicine, it is as vital as ever that we play an increasingly active role in patient care.

Philippe Tanière is Consultant Histopathologist at Queen Elizabeth Hospital and Honorary Senior Lecturer at the Medical School, Birmingham, UK. Matthew Evans is Specialist Registrar at University Hospitals Birmingham, UK.

Resolving the Pathologist's Dilemma

Patient samples are precious – as is the information they yield. Current assays present a choice between spatial information, precision, and plex. But compromise may not be necessary; digital spatial profiling (DSP) allows highly multiplexed, multi-analyte quantification of RNA and protein from FFPE tissue. With a non-destructive approach, you can do more with less.

By Dr. Alessandra Cesano, Chief Medical Officer, nanoString, Seattle, WA, USA.

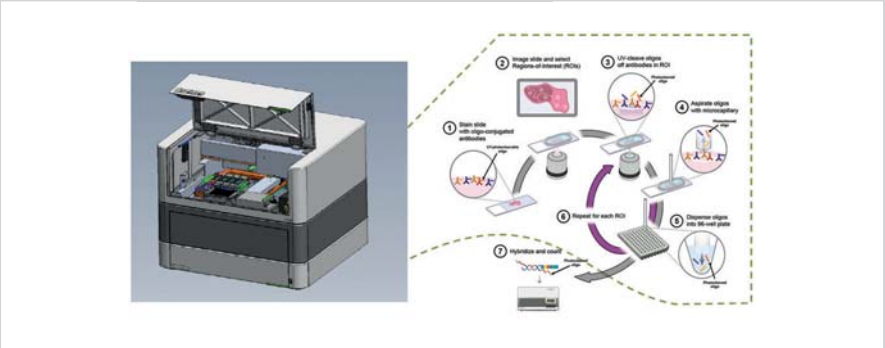


Figure 1. An overview of the DSP workflow demonstrating how the DSP instrument prepares multiplexed assays from a single tissue biopsy slide.

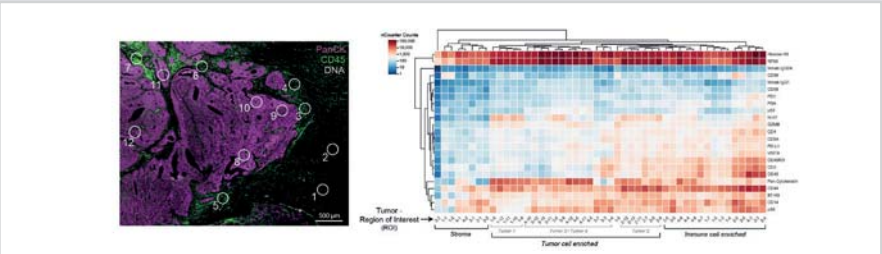


Figure 2. Immune characterization of a colorectal cancer excisional biopsy by DSP (1). Tumor tissue (left) was fluorescently stained for pancytokeratin (PanCK), CD45 and DNA. Different regions of interest were selected for high-plex protein expression profiling (right). Matching expression levels to regions of interest allows tumor and microenvironment characterization and, potentially, treatment personalization.

Understanding cancerous tumors and their microenvironments permits more detailed and comprehensive research, potentially leading to more accurate prognoses, personalized treatment decisions and disease monitoring.

The problem

The tumor microenvironment plays a vital part in cancer's progression and responsiveness to treatment – which makes it a key target in both the laboratory and the clinic. Typically, imaging studies are used to understand the microenvironment in biopsy samples, but the techniques used are fraught with difficulties. They offer limited dynamic range, poor precision, and require significant time and effort to analyze a single target.

Conversely, RNA or protein expression profiling assays are quantitative, precise, and can be performed at high plex. Due to the "grind and bind" nature of the assay, however, all of the valuable spatial information is lost.

For precision oncology, that is not good enough. Researchers need the ability to quantitatively evaluate and characterize RNA and protein while maintaining spatial information. This allows them to establish the overall morphology of the tumor tissue, and conduct further high-plex profiling if necessary. Observing the spatial distribution of abnormal cells – and their subtypes – allows researchers to determine whether a tumor is benign or malignant, immune "hot" or "cold," and whether or not current treatments are effectively controlling or eliminating disease. Without this information, it is difficult for health care teams to make appropriate diagnostic, prognostic and treatment decisions.

But this view is difficult to obtain with imaging or profiling capabilities alone – and all of these assays consume precious patient samples. This presents pathologists with a dilemma: what is most important to preserve? Is it sample, spatial information, or high-plex target information?

With laboratories already under stress and the need for pathology services only increasing, is there a way around this dilemma? What pathologists need is a practical way to overcome these challenges and provide maximum information from precious tissue samples.

challenge in the event of a difficult biopsy or low tissue volume, and even in situations where samples are easily obtained, each individual section involves a time and labor investment on the part of the pathologist. With laboratories already under stress and the need for pathology services only increasing, is there a practical way to overcome these challenges?

The solution

Digital spatial profiling (DSP) is a novel platform, based on analyte barcoding technology, that enables the spatially resolved characterization of RNA and protein in a highly multiplexed, nondestructive assay. The instrument is capable of examining up to 800 analytes at once, and RNA or protein can be profiled on a single FFPE slide section (see Figure 1).

Once the analytes of interest have been quantified, the counts can be mapped back to their original location on the tissue sample, providing spatial resolution for the quantitative data (see Figure 2).

Reference

1. *N Confuorto, Presented at the 4th Digital Pathology Congress; November 20, 2017; London, UK.*

NextGen

Research advances
New technologies
Future practice

A Sketch For Success

A new rapid DNA re-identification technique can make cell line authentication and other processes faster, easier and cheaper

By Sophie Zaaijer

You've just published a paper in a highly ranked cancer research journal. It's a project that has taken years of your life and hundreds of thousands of dollars in funding. It has been challenging, exhausting, and exciting – and it's just the first step on the road to a much broader answer; maybe even a cure.

But there's a problem. Another laboratory, attempting to reproduce your results, hasn't been able to. Why? Extensive testing points the finger at the cell line you used in your experiments – a cell line you use in almost all of your work. Suddenly, you're faced with questions: Is that cell line really what you think it is, genetically? Can you trust it? And if not, are your results still meaningful?

Cell line authentication is a vital part of medical research – and yet, because of the time and effort required by current methods, it's often postponed or overlooked. This, and many other problems across research and clinical boundaries, could be solved by the application of a rapid DNA re-identification method. That's why my colleagues and I developed MinION sketching (1) – a new way of using existing technology to make DNA identification fast, cheap and manageable.

At a Glance

- *Cell line authentication is a major problem in life sciences research – not because there are no tools, but because they are tedious and time-consuming*
- *Rapid DNA re-identification not only speeds up cell line authentication , but could also find utility in disease diagnosis and forensic investigations*
- *A new method, MinION sketching, uses a portable DNA sequencer and custom software to provide a quick, affordable DNA identification method*
- *The technique requires further development, but has the potential to solve a wide range of laboratory, clinical and other scientific problems*

"The ability to take a DNA sequencer anywhere to analyze nature outside the walls of a specialized laboratory is a true game changer."

Why DNA-based rapid identification?

It was the idea of a portable DNA sequencer that really triggered our imagination. The ability to take a DNA sequencer anywhere to analyze nature outside the walls of a specialized laboratory is a true game changer. Imagine being able to re-identify individuals at crime scenes almost in real time – using this knowledge to prevent a perpetrator from striking again. DNA fingerprinting currently takes days, between sample transport, queuing, preparation, and running the DNA sequencing devices and interpretation software. A portable DNA sequencer would solve this problem, we thought, allowing us to re-identify DNA samples on-site.

While working on developing robust methods to employ portable DNA sequencers in the field, we realized that the method we had devised (see "MinION Sketching") could also make a difference to a long-standing problem: periodic cell line authentication in research labs. Cell lines derived from patients are crucial for the research of specific diseases. In the lab, such cells are carefully studied to understand the

molecular mechanisms behind the illness. Although each cell line behaves a little differently on a molecular level, they often look very similar under the microscope. As a result, accidental contamination, mislabeling or label swapping is an unfortunate and hard-to-track inevitability; for scientists who may spend years figuring out the underlying molecular mechanisms of a particular diseased cell line, that can lead to disaster.

The lack of cell line authentication is a long-standing problem in disease research and, because it results in irreproducible research, a major cause of wasted research money. However, the problem is not because of an absence of available tools; rather, it's because of the time and effort it takes to use those tools. Our method enables rapid, on-site identification via DNA fingerprinting, an approach borrowed from the forensic sciences as an excellent method to help authenticate the origins of cell lines. The methods currently offered by third parties or done in-house are long and tedious; our method, in contrast, allows rapid checking by DNA fingerprinting as part of the standard laboratory toolkit. And that could reduce scientists' resistance to regular testing. Not only will this be a step toward making research with cell lines more efficient, but it will also eventually bring us closer to cures for a multitude of diseases.

How it works

When we started our project, the MinION portable DNA sequencer had a high reading error rate. For approximately every 10 nucleotides it read, one was wrong. The difference between individuals is about one in every 1,000 nucleotides – so a 10 percent error rate was unacceptable! We needed to find a way to bridge the gap and still be able to use some of the MinION data. To that end, we developed a weighing method in which we determine the probability that a given nucleotide reading is an error and then consider the probability that we might see it in the general population. Of course, the less commonly observed a nucleotide is in the population, the more informative it will be in attempting to trace a sample back to a single individual.

The MinION reads DNA in real time, so each informative nucleotide that comes off the DNA sequencer is another piece of evidence. The evidence

MinION Sketching

The method, which requires a MinION portable DNA sequencer and custom software, involves two steps.

1. We sequence random strings of DNA from a given cell line or sample. From these random strings, we select individual single nucleotide polymorphisms (SNPs) that vary from person to person and can thus be used as identifiers.
2. We run a Bayesian algorithm that compares these SNPs with the database of genetic profiles on file. For cell line authentication, that might be a database containing the genotypes of every cell line used in the laboratory; for person re-identification, it might be one that contains the sequences of individuals in the relevant population. As the software cross-checks each variant, it updates the probability of a match until it narrows the options to a single reference profile

sequentially updates the posterior probability of a match to one reference file in the database. If the MinION sketch does not match any entry in the database, all posterior probabilities will stay low and the method won't yield a match – but if the probability of a match is high, the method will flag that file in the database for review. We have tested extensively for false positives and optimized our method so that we don't run into such problems. The re-identification opportunity is only as good as the database, of course – as with all forensic methods – but if the database doesn't contain a corresponding reference file, there will be no match.

"It's not a 'blue-sky' future projection – it's already here. People can begin MinION sketching right now!"

The future of MinION sketching

The technique has a number of applications, both within and beyond the walls of the laboratory.

- *Basic research*
  Cell line authentication isn't the only use for MinION sketching. Because it doesn't selectively amplify specific stretches of DNA like other methods, it allows for the identification of pathogens infecting those lines. For instance, *Mycoplasma* contamination often affects laboratory cell lines and can be challenging to detect.
- *Forensic science*
  Our method can be used as a tool for rapid re-identification of individuals after mass disasters. After such events, family members are understandably keen to locate their loved ones. They can contribute by sharing their SNP reference files with the forensic team to facilitate matching and re-identification analyses. The samples can rapidly be checked on-site, even in remote areas, letting families be reunited with their missing members – an amazing advancement. 2017 was the 20-year anniversary of GATTACA – a movie that predicts a future in which identities are verified not by cards or photographs, but by DNA fingerprint matching. With MinION sketching, such a future may not be far off. Will we soon give up our passports in favor of our DNA?
- *In the clinic*
  DNA fingerprinting is an easy way to track clinical samples. It can allow the identification of infectious pathogens, too, as well as any antimicrobial resistance markers that might affect treatment decisions (2). I also foresee its application in organ transplantation verification; immediately before surgery, the patient and the donor organ can rapidly be authenticated as a last check for a correct donor-recipient match.
- *Other applications*
  There are many opportunities to use our method in other fields. For instance, we have investigated its use in dog and cow re-identification. This enables high-resolution tracking of individual species. Instead of a microchip system, owners can be reunited with their lost dogs via a quick DNA test. Cows can potentially be traced from farm to table. If we can do that, what about tracking high-value horses? Cats? Animals at risk of poaching (2)? The list is endless!

The best part? In many of these examples, it's not a "blue-sky future" projection – it's already here. People can begin using MinION sketching right now! For cell line authentication, for instance, the first task is to compile a good reference database of all cell lines present in the laboratory. Then, it's just a matter of a few easy steps:

1. Set up of our Person ID pipeline on a computer (see "MinION Sketching"). This requires some command-line knowledge.
2. In the wet lab, extract DNA from each cell line.
3. Perform a MinION library preparation of the DNA.
4. Sequence the DNA and generate a MinION sketch.
5. Run the Person ID pipeline and analyze the matching results against your compiled database.

MinION sketching is a "new kid on the block" for a vast number of applications. My colleagues and I are still working to develop it, of course, but it's already available for use – and it's a simpler, cheaper alternative to many of the current options. It's my hope that, as both the technology and the availability of reference genomic data advance, researchers and laboratory medicine professionals will be able to work with confidence, knowing that their cell line – and their science – is trustworthy.

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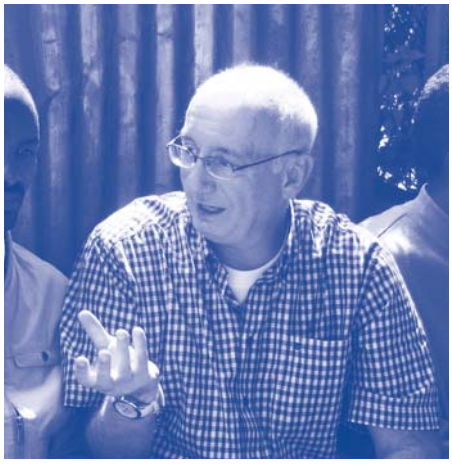

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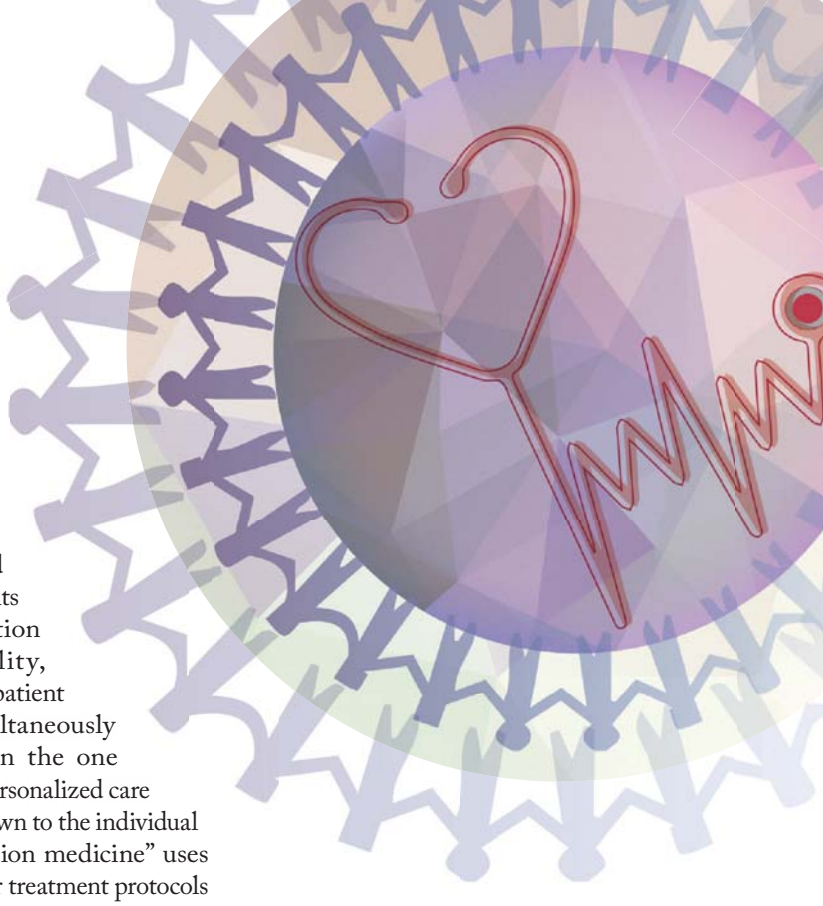

No Longer Just the Doctor's Doctor

The changing face of population health is revealing the essential role of the pathologist as a curator of population health

By Sandip SenGupta

When attempting to dispel the myth of the pathologist locked in a basement – friend only to the microscope and the tissue section – many of us turn to examples of patient interactions. It's certainly true that there is a place for us in face-to-face conversations with patients, just as there is a place for us in medical education, and in community outreach. But these interpersonal interactions aren't the only place outside the laboratory where our skills have value – and one oft-overlooked arena is, in my opinion, among the most important places where we can make a contribution; I see us as curators of health – not only for individual patients, but for entire populations.

At a Glance

- *Pathologists can combine their diagnostic and interpersonal skills to become curators of population health*
- *Unmet needs in utilization management, patient tracking, and telemedicine can all be met by pathologists who choose to take on such tasks*
- *We can also help educate other healthcare providers to optimize patient care on a large scale*
- *New technologies mean that modern population health management is at the very beginning of its evolution – and pathologists have a vital and exciting role to play going forward*

The population's doctor

In healthcare today, there are two somewhat parallel tracks, each focused on the same elements of the value equation – improving quality, access to care, and patient safety, while simultaneously reducing costs. On the one hand, increasingly personalized care narrows the focus down to the individual patient; this "precision medicine" uses genetic tools to tailor treatment protocols to improve the chances of a successful outcome and reduce unnecessary side effects. On the other hand, "population health" aims to manage and improve quality healthcare outcomes – not for specific individuals, but for groups in a community or a region. Population health also makes increasingly frequent use of genetic tools, such as genomic registries, to accomplish its healthcare goals.

Population health management offers us, as pathologists, a wonderful opportunity to use not only our diagnostic abilities, but also the teaching, administrative, and leadership skills that we have honed during our medical practice. It allows us to extend our sphere of influence well beyond our traditional "institutionalized" domains (in the hospital or private laboratory setting) into regional communities of care. It is an excellent opportunity to work collaboratively with other healthcare providers to ensure that at-risk populations are properly and regularly screened for diseases such as diabetes, cervical cancer and heart disease. As healthcare administrators and government decision-makers increasingly shift resources out of traditional acute care hospitals and into ambulatory care organizations (such as standalone surgi-centers, endoscopic clinics, or mother–child wellbeing centers), how can pathologists avoid becoming marginalized – both in terms of their remuneration and their interactions with other medical colleagues?

The answer: by stepping up to the plate – demonstrating our value to our healthcare ecosystem and by helping to lead the way through the digital transformation of our healthcare enterprise. As resources for healthcare continue to shrink and demand continues to soar, pathologists can show how targeted test utilization management (using sophisticated "big data" analytics) and clinical decision support tools, along with emerging artificial intelligence technologies, can save money and improve care for patients across the board.

A new kind of patient care

The beauty of population health management is that the need for direct contact with patients is even less of a barrier than it might be for pathologists involved in caring for individual patients. All physicians – whether they specialize

in laboratory medicine, nuclear medicine, or emergency medicine – can contribute meaningfully in their respective disciplines to the bigger picture of health across populations. Indeed, the skill set required of such physicians is less focused on diagnostic abilities and more focused on a strategic mindset and organizational skills related to the transformation and integration of health systems. There are far more diverse ways to help our patients through the lens of population health than one-on-one conversations (though, of course, those remain valuable). Instead, or in addition, pathologists can tackle issues such as improving access to care, and maintaining the continuity of care as patients move – either vertically through the spectrum from primary to tertiary care, or horizontally amongst family physicians, nurse practitioners, and midwives in their community. After all, who is better placed than a pathologist to continually track a patient through the system using only their lab test results and medical records?

Pathologists, especially those with more generalist training in both anatomic and clinical pathology, are well poised to use their knowledge and skills in laboratory information systems (LIS) to guide the interoperability of hospital information systems (HIS) at the regional level. After all, it is often said that greater than 70 percent of clinical decision-making is based on patients' laboratory information! Where better to start in population health management than in data mining the LIS and linking it meaningfully with other clinical data repositories? Not only will this help track patients and ensure continuity of care, but this kind of data analysis can also lead to new insights into improving patient care pathways in the community.

There is no shortage of potential tasks to take on in population health, but there is often a serious shortage of skilled managers, directors, and leaders to engage in important projects. By raising our hands and offering to lend our expertise, we as pathologists can quickly demonstrate our value. But be warned – many such forays into population health may not have fee codes for professional reimbursement. In the early stages, we may have to accept that it is simply the right thing to do for other reasons, although it may be a "loss leader" in terms of income (unless you are lucky enough to be recognized specifically for your efforts, perhaps through an administrative stipend or other type of sessional remuneration). If you are willing to take on such a task on behalf of your patients without requiring payment, it may offer a pleasant part-time diversion from "pushing glass" all day long… Get out there and mix with other healthcare providers; let your creative juices flow through a cross-fertilization of ideas and, together, you may be surprised by the unparalleled joy of making a difference to your community!

Bridging care gaps

The most obvious unmet need that we can tackle is in laboratory test utilization management, especially in primary care. Many family physicians and general practitioners are simply overwhelmed by the avalanche of new information that crosses their desks every month. And yet, most laboratory tests are ordered by primary care providers, not specialists. These doctors are often the gatekeepers to a patient's health, and it is our job to help assure patient safety and prevent harm by avoiding unnecessary laboratory tests – and the potential deleterious downstream consequences of chasing abnormal, yet ultimately unimportant, results.

Improving and integrating healthcare across multiple providers is another opportunity for pathologists interested in population health. For example, optimal diabetes care may require that certain tests (such as HbA1c) are not over-utilized, whereas others (such as eGFR to monitor renal function) are used often enough. Similarly, through the horizontal continuum of care, patients discharged from hospital without laboratory test results are at risk of being lost to follow-up, if they can't be recalled for treatment of any abnormalities discovered after their departure. Pathologists can even help track the screening and monitoring of high-risk populations. Screening for cervical cancer using human papillomavirus testing is an example; whereas previously we might not have known which patients or populations were receiving adequate testing, nowadays, sophisticated data analytics can link patient demographics with test-ordering patterns and results.

Pathology as a linchpin

Pathologists have a key role to play in almost every aspect of population health. Take, for instance, the integration of laboratory information across the continuum of care. We can participate in multidisciplinary forums, join committees, or even lead task forces whenever the interoperability of health information is being considered, or when a new HIS

is being purchased. We can engage in innovation and technology initiatives in our local regions – in fact, many of us already do so by virtue of telepathology and digital imaging. In my region, for example, we are looking at implementing relatively simple digital cameras attached to microscopes in rural community hospital laboratories. The goal? To capture abnormal blood smears and Gram stains for rapid diagnosis by specialists at an academic health sciences center 100 kilometers away. In general, I think that patients in small, rural, underserved communities stand to benefit the most through this type of population health initiative. It means that their access to care stays close to home, but diagnosis –thanks to advances in technology – may take place hundreds of kilometers away.

Advances in digital imaging, and its much lower associated costs, are revolutionizing pathology in uncountable ways. It might be through remote (yet faster) diagnosis. It might be through sophisticated medical data analytics for test utilization management and clinical decision support. It might be through collaborating with clinical colleagues to improve patient outcomes – for instance, continuously monitoring patients with heart failure by electronically integrating lab results from point-of-care devices in their homes with their physiological data to prevent the need for readmission to the hospital. Regardless of how your particular region chooses to implement technological advances, there is likely to be a role for you in the patient care pathway.

Pathologists can also demonstrate their value to disease management by guiding clinicians in monitoring treatment compliance in the ambulatory care setting. Examples that come to mind include chronic kidney disease (ensuring that patients do not end up on dialysis or suffer from other complications), and heart failure. In my region, pathologists work closely with anesthesiologists to identify (through brain natriuretic peptide testing) patients in the community who are at high risk of postoperative cardiac complications of elective major surgery. Hepatitis disease management is another example where pathologists can play an important role by alerting clinicians in the community to high-risk patients. Ideally, we would implement a system of automated alerts on providers' ordering behavior – something along the lines of, "Your patient has not had ALT testing performed in last six months; please consider testing at this time." A pathologist would certainly be a useful source of information on appropriate testing during the development of such an alert system.

Growing alongside technology

Point-of-care testing, the increasing simplicity and robustness of genomic testing tools, and the miniaturization of technologies are just a few examples of how innovation will change the face of healthcare. There is a growing paradox in that the location of diagnostic testing will grow closer and closer to the point of care with smaller and smaller samples (such as saliva or blood for liquid biopsy) – yet the information generated from such test results is increasingly nuanced and challenging to interpret. Much of it will require specialized expert opinions from well-trained molecular pathologists, meaning that the need for pathologists "in the field" may be far greater than ever before. Gone will be the days of large, factory-like private laboratories where the pathologist has little contact with the practicing clinician. In a virtual age, our value may be best delivered through cyberspace, offering opinions in real time to patients and healthcare providers.

Here, too, our contributions to utilization management can make a difference. Many laboratory tests sound very similar to one another, but are very different indeed – take, for instance, Factor V Leiden versus Factor V. Most of these tests are ordered in the community, so there are opportunities for pathologists at a macro level to follow trends of test utilization and bring anomalies to the attention of regional health authorities. The extent of test duplication, retesting at another facility, and discordant test results are all worthy of scrutiny. Consider expensive genetic testing, where our contributions toward the development of registries (such as those available for hemochromatosis, Factor V Leiden, or HLA-B57) can be very helpful. We can also contribute to the development of automated, electronic health record-based notifications to hospitals in our regions to intervene and cancel unnecessary genetic tests.

We can assess the reliability of one test assay over another, especially for the new tests introduced onto the market, by reviewing data at the regional level. If we do this in a timely fashion, we can make the necessary changes before it's too late. Of course, there is always a risk of overcorrection when making procedural changes; however, a properly designed and resourced system, with checks and balances that include regular monitoring of metrics and multidisciplinary forums for communication with stakeholders, can negate any potential issues.

Population health is a massive, multifaceted science, and pathologists are only beginning to scratch the surface of our role in its evolution. We tend to think of ourselves as caretakers of individual patients – or even of individual subspecialties and sample types. And so it is not easy to recalibrate our thoughts to consider ourselves as large-scale medical practitioners; many pathologists may not realize how much population health work they are already doing, or how much they are capable of doing within the parameters of their current practices. Of one thing, I am certain: we have a unique and valuable contribution to make to population health, and it is up to us to ensure we are ready and willing to make it.

Sandip SenGupta is a Professor at Queen's University, Kingston, Canada.

The Beauty of Glass

In an increasingly digital world, the AMR Club keeps the educational and aesthetic value of the microscopy slide alive

Ivan Damjanov interviews Saul Suster

Saul Suster is Professor and Chairman of the Medical College of Wisconsin's Department of Pathology, and an unstoppable force in the world of pathology. Although an internationally known pathologist, a scientist, a prolific medical writer and the author of several well-known pathology textbooks, he decided he was not busy enough – so he founded, and is now the president of, a unique pathology club known colloquially as the "AMR Club." I myself joined the club in 1996 and have attended several of its international meetings, including the June 2017 gathering in Krakow, Poland. During the meeting, I asked Saul if he would tell us a little more about his unique pathology organization – a club that has, over the last two decades, gained a global following and to this day offers postgraduate education in a traditional, yet novel, form.

At a Glance

- *The AMR Club is a unique community dedicated to the exchange and discussion of glass slides*
- *Members tend to be highly regarded academic pathologists, but the club's benefits extend far beyond its membership*
- *Many of the club's cases have been used as teaching aids, in textbooks, or even as reference slides to compare with pathologists' routine work*
- *The club also runs meetings and encourages the broader pathology community to appreciate the beauty and the value of a classic H&E slide*

Why the "AMR Club?"

When I started the club in 1991, I named it in honor of my most influential teacher and mentor, Arkadi M. Rywlin – "AMR." Arkadi Rywlin was born in Danzig (presently known as Gdansk), Poland, and trained as a pathologist in Geneva and later Chicago. Eventually, he settled in Miami to become Chairman of Pathology at the Mount Sinai Medical Center of Greater Miami and Professor of Pathology at the University of Miami School of Medicine. He was one of the most prominent hematopathologists of his generation, even authoring a textbook on the histopathology of the bone marrow (1). He was also an encyclopedic pathologist with a privileged eye for surgical pathology, but none of those counted as his greatest talent – that title was reserved for his passion for teaching.

He trained hundreds of residents with a teaching style so unique that his students had a term for it – "Rywlinian." Arkadi Rywlin was an iconoclast who believed it was our personal responsibility to question everything – to pass all information through the filter of our intellect before accepting it as gospel. He taught by example and truly motivated his students to think. "Always exercise your largest muscle," he would say – and by that, of course, he meant the brain. His prowess at the microscope was awe-inspiring, and to be a student of his, listening to his rationale for arriving at a difficult diagnosis was always a riveting experience. His was the only residency program where the Chairman of the department personally spent three hours of every day at the microscope with all his residents, discussing cases and expanding their minds through

his challenging questions and constant prodding. Training under him was a real privilege and the highlight of my career. Is it any wonder that, when founding a club designed to encourage pathologists to think, I immediately thought to name it after Arkadi Rywlin?

Tell us some of his legendary "Rywlinisms…"

My favorite aphorism, and the most important lesson I learned from him, is that "the best lie is the truth." He would often say that at the daily "show and tell" sessions he held at an 18-headed microscope in his office. During those three hours, he would review all of the interesting and challenging cases of the day with his staff and residents – and any time a resident tried to fib through questions instead of simply saying, "I don't know," he would resort to that phrase. It's far better to admit a lack of knowledge and learn something than to hide it and end up no better off than you began!

Another favorite saying of his was, "We try not to worship the routine here." What he meant was that we should always strive for excellence, innovation and constant improvement. However, one of his most important sayings was: "A paper cannot defend itself." He didn't want us to take anything for granted or to believe everything that we saw printed on a page; instead, he wanted us to constantly process and analyze information. He thought that the ability to challenge dogma was the optimal way to advance our knowledge.

What are the club's goals?

The main goal of the AMR Club is to provide a platform for its members to exchange interesting and challenging cases in a friendly, noncompetitive spirit. The idea is to share and broaden our knowledge and experience in the different aspects of anatomic pathology. The organization's official name – the International Pathology Slide Seminar Club – gives a better hint as to its functions. First of all, it is international; its purpose is to promote a discourse between pathologists from all parts of the word. If we had more histopathology exchange clubs and other forms of person-to-person contact across the globe, maybe we wouldn't have wars and conflicts – but that is a topic for another discussion. True to its name, the club currently has 45 active members spanning the globe from North and South America to Europe, Asia and Australia; all are academic pathologists who share a passion for diagnostic surgical pathology and enjoy taking on challenging and difficult cases. The second key word is pathology; the club was founded by pathologists, for pathologists, in honor of a pathologist, and to promote pathology. We also use the word slide, which obviously refers to microscopic slides – not digital images, but the real, tangible glass itself. The hematoxylin and eosin-stained slide forms the backbone of our specialty and represents the essence of our profession. I learned to love H&E glass slides the same way as Arkadi Rywlin did. And that's why the club is based on an actual exchange of representative histologic glass slides among its members, rather than the sharing of images or other media.

What do you do?

First and foremost, the club is built on an active exchange of glass slides and comments contributed by members to each other's cases. Approximately three times a year, I receive a set of H&E recuts from the cases being contributed. I collate them into sets of slides and mail them back out to all members for their review, at which point I also ensure that the information for the cases is posted on the AMR website. Each member then reviews their set of slides and sends their comments, queries and opinions to me by email. Finally, I gather all of these thoughts into a single document and post it on the website (amr-seminar.org) for everyone to read.

"I would estimate that about 50 publications have been inspired by interactions that first took place within the context of the AMR Club."

After the initial success with our internal exchange of cases, it was suggested by some members that, given the cases' high education value, perhaps we could share them with a broader audience. This led to a proposal that we organize an actual seminar in which to present the cases to the public. The first International AMR Slide Seminar Symposium was hosted by one of our members, Michele Bisceglia, in 1991 in San Giovanni Rotondo, Italy. The event was a great success, with broad participation by pathologists from Italy and several other countries, and the positive outcome encouraged us to make further plans. So far, there have been 11 international events, including two presentations at International Academy of Pathology meetings. We've held seminars in Italy, the Czech Republic, Mexico, Australia, Turkey, Sweden, Israel, Japan, South Africa, Slovakia, and Poland, with one in Croatia scheduled for May of 2018.

Nowadays, our events are designed as extended slide seminars in which members of the club present short cases. We use those cases as a platform to discuss the different entities and controversies in surgical

The location of the 8th AMR Club in 2015: Bratislava, Slovakia.

pathology and present a state-of-the-art discussion on the various topics. About 70 to 90 cases are presented at each three-day meeting, and the first 120 attendees receive not only a detailed syllabus, but also a complete set of H&E glass slides from all the cases being presented. The meetings have been enormously popular (with nearly 2,000 pathologists from 30 countries having attended so far) and serve as an opportunity for the AMR members to bond with one another, as well as take an enjoyable trip with their families.

One of our former club members, John K.C. Chan, was the founding editor of Advances in Anatomic Pathology. Thanks to him, the journal has a section devoted to presenting selected cases by members of the club. Juan Rosai, a founding club member, has also digitized the entire collection of glass slides contributed over the years in conjunction with the United States and Canadian Academy of Pathology and incorporated it into the Juan Rosai Collection of Surgical Pathology Seminars (2).

How do you benefit from the club?

More than anything, all of the club members enjoy the discussions about the slides. They aren't the only benefit, though. Many of us use the slides we encounter at the AMR Club to teach our residents and fellows, and we widen the net by showing the slides to our colleagues as well. That way, the entire pathology community has the opportunity to participate in the conversation. Some of us use the club's slides as references, comparing them with ones we encounter in our daily routine service. Many of them have been photographed for teaching purposes – some have even ended up as textbook illustrations! And they stimulate further study, as well. I would estimate that about 50 publications have been inspired by interactions that first took place within the context of the AMR Club.

"I'm very proud that we have been able to provide engaging, educational content..."

The Club is a not-for-profit voluntary organization; membership is free, but by invitation only. Most new members are recommended by existing ones, based on their suitability to participate and the quality of their potential contributions. Most – possibly all – of our members are academic pathologists, many of whom are chiefs of service at their respective departments, as well as recognized experts in their fields. Other than that, the only requirement for membership is active and enthusiastic participation and regular contribution of cases and comments.

Of course, it's not only members who benefit from the AMR Club's operations – and I'm very proud of the fact that we have been able to provide engaging, educational content both within and outside the boundaries of our club membership.

Saul Suster is Professor and Chairman in the Department of Pathology and Laboratory Medicine, Medical College of Wisconsin, Milwaukee, USA.
Ivan Damjanov is Professor of Pathology at the University of Kansas, Kansas City, USA.

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Landing the Lead Role... in Pathology

Sitting Down With… Elizabeth Montgomery, Professor of Pathology, Oncology and Orthopedic Surgery at Johns Hopkins School of Medicine, Baltimore, USA

How did you first get into pathology?

I didn't know that pathology existed until I went to medical school. I wanted to be an actress, but my parents said that my choice was not realistic (too short? glasses too thick? use too many swear words?). Instead, I went to university and studied chemistry! Medical school followed naturally; my plan was to study obstetrics and gynecology – but then we reached the pathology course and it was love at first slide. Even though I considered the first-year normal histology course to be the stupidest thing I had ever encountered, I couldn't stay away. Steve Silverberg, one of the faculty members at my medical school, made pathology look cool and fun… (I had to go back and figure out histology. Oops.)

Steve was a real inspiration to me in medical school. Because I joined the military to pay for my education, my residency was in uniform at Walter Reed Army Medical Center in Bethesda. At the time, the Armed Forces Institute of Pathology (AFIP) was still in its glory days and right next to Walter Reed, so I had the great honor of learning from wonderful morphologists, including Tanya Tavassoli, Dennis Heffner, Glauco Frizzera, and many others.

What led you to gastrointestinal and soft tissue pathology?

As a resident, I loved soft tissue pathology very much. I had the privilege of working with Sharon Weiss for a little while as she transitioned to the University of Michigan. I also loved the time I spent working with the orthopedic surgeons at Walter Reed. Upon graduating from my residency, and after being sent to an Army community hospital for a time, I was ultimately assigned to the AFIP soft tissue branch and had the opportunity to work alongside Jeanne Meis and Franz Enzinger, who were both spectacular morphologists. It was a fantastic experience.

When I left the Army, I took a job at Georgetown University. They wanted a gastrointestinal pathologist, so I began educating myself. Although I had done some projects on *Campylobacter pylori* (now termed *Helicobacter pylori*) as a Walter Reed resident, in collaboration with my gastroenterology colleagues, there was still a lot to learn! It was a bit of a challenging process, but I eventually caught on. Because of that experience, I ended up moving to Johns Hopkins in 1999, where I have remained ever since.

"We reached the pathology course and it was love at first slide."

What are you most proud of in your career?

I am not a "hot stuff" scientist, but I do feel proud of the fact that I have been able to help with the success of many former fellows – some of whom *are* "hot stuff" scientists! I am also very proud of having written the original descriptions of a few entities in surgical pathology, and of a small body of textbooks that I hope are of value to my colleagues.

Of the 15 textbooks I have authored, my favorites are the biopsy interpretation books in the Wolters Kluwer Biopsy Interpretation Series. The gastrointestinal pathology one was updated to its third edition not long ago – a big effort, because each textbook not only has images in its main text, but also includes a large cache of online images and quizzes. I was also recently appointed Editor in Chief for the fifth edition of the renowned AFIP Atlases of the American Registry of Pathology – the "tumor fascicles" series. My colleagues and I are really excited about the fifth series as we wrap up the fourth (the Intestines fascicle, to which I was a contributor, was released in late 2017). We have been doing a lot of preliminary work to take advantage of new technologies and digital options for the new series, which will have online content, including virtual slide boxes!

In addition to working on pathology texts, I also enjoy giving invited seminars. Over the course of 2017, I had the opportunity to give about 50 individual presentations in 18 different venues. It's fun to travel around the world and meet colleagues in a variety of different places. One of my favorite organizations to work with is the Sociedad Latinoamericana de Patología; I have been learning Spanish over the past few years, so I enjoy giving lectures in my (still imperfect) Spanish.

My favorite activity of all, though, is looking at my daily consultation cases with our fellows. Colleagues in the trenches share the most amazing lesions and do such a wonderful job that it is a real pleasure to see these "curated" cases.

If you were not a pathologist, what would you have been?

An actress, which probably means a waitress. Maybe I could have found an expensive restaurant with great tips…

With so many challenges and responsibilities, how do you manage everything?

I just twinkle my nose and it all gets done, of course!

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