JANUARY 2016 # 15

the Pathologist

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He's inspired and educated generations of pathologists, and Manuel Sobrinho-Simões doesn't plan on stopping

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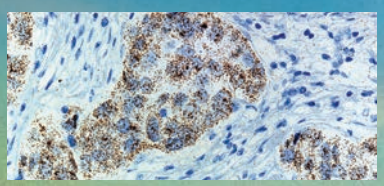

HER2 expression in human breast cancer FFPE tissue using RNAscope® 2.0 HD Reagent Kit-BROWN

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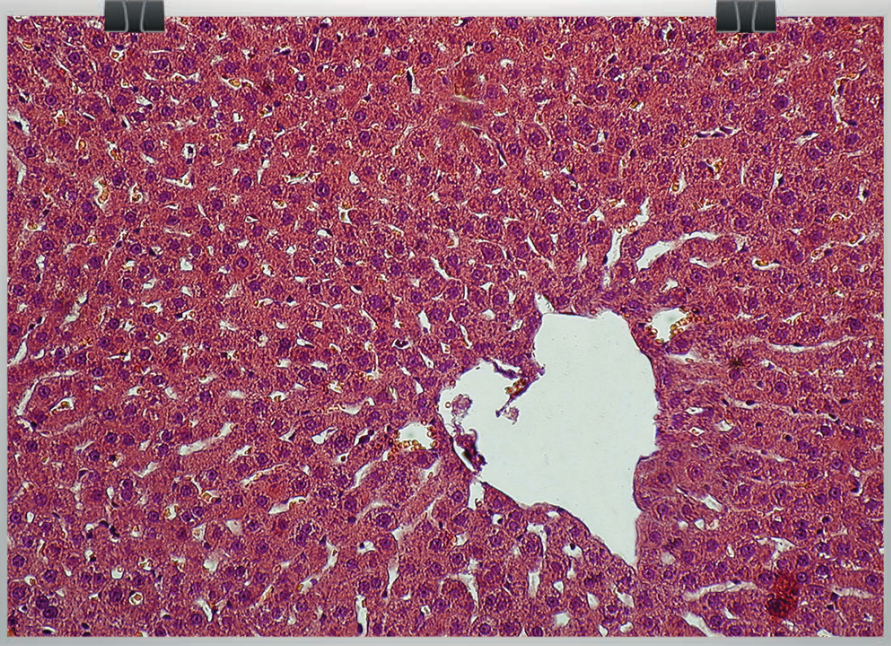
Image of the Month



Passionate pathologist IHeartHisto (ihearthisto.com; @ihearthisto) creates and curates interesting pathology images from around the Internet. He and many like-minded pathologists use hashtages like #PathArt to find one another and share the best – or funniest – examples of their work. This image shows a central vein of the liver.

See more #PathArt and read about its value to the pathology community on page 45.

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2015 Winners
Andreas Seidel-Morgenstern (left)
and Peter H. Seeberger (right),

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Our Technological Crutches

Is almost complete reliance on technology a good thing?

At the time of writing, my smartphone was continuously alerting me to the fact that "Tim Peake has begun the first ever spacewalk by an 'official' British astronaut." It may seem cynical of me, but I did wonder why there was so much press coverage in the UK on Tim's space expedition journey, which had started weeks in advance of "lift off". Was it worthy of continuous news alerts? In any case, it got me thinking about our reliance on smartphone technology to keep us updated. Personally, I can't help but look at my phone every time a news notification pops up. You never know what it might say or how the news could affect you. But is our craving for instant information a good thing? And are those who aren't permanently attached to their phones missing out?

I certainly don't know how I would get by, long-term, without my smartphone. So when this month's cover feature interviewee (and our Power List Number One) Manuel Sobrinho-Simões admitted that he has never owned one, I was surprised. In an era when the influence and use of digital technology is increasing substantially, including in pathology, how do you survive without it? But that's not to say Sobrinho-Simões is behind the times; he and his team in Portugal were using molecular pathology techniques and conducting truly translational research back in 1989. In fact, they made numerous genetic discoveries that were later verified (some eight years later) with the most sophisticated molecular techniques available at the time. So perhaps what's more important than technology is to be able to predict trends and to use the knowledge and the tools that you have to stay ahead of them – wherever that's possible.

Don't get me wrong, I continue to be wowed by new technologies – what developers are managing to achieve is incredible. Take the game-changing innovations in molecular diagnostics. Though the optimum use of these techniques in clinical pathology is still be up for discussion, the positive impact that technological innovation has made in this field is undisputable.

Thinking more broadly, I do wonder what impact some less essential innovations might have on society and the way that we interact with each other – as humans. Only today, I read a news story of a chef who banned the use of mobile phones in his restaurant. I thought it was a great move, but it sparked very strong criticism from many members of the public...

As Sobrinho-Simões suggests, perhaps the key is to find a balance between traditional methods and technological innovation. But whether that is the right approach for pathology, only time will tell.

Fedra Pavlou
Editor

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?

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Another Blow for Fingerprick Diagnostics

The demand for simple, point-of-care fingerprick testing is growing, but could a study highlighting drop-to-drop sample variation throw a spanner in the works?

DNA Medical Institute. Genalyte. And perhaps most famously (or infamously), Theranos. What do these companies have in common? All are developing technologies that rely on a simple fingerprick blood draw to provide quick and easy diagnostic testing. But now, along with the recent questions raised over what Theranos "nanotainer" technology is truly capable of (1), comes a study that casts doubt on the accuracy of results gained from fingerprick blood, regardless of the technology used to obtain and process it.

Two bioengineers from Rice University, Texas, USA, decided to investigate the drop-to-drop variation in results when using fingerprick blood for whole blood count testing. And their initial findings could spell trouble for test developers: the amount of variation in repeated samples of fingerprick blood was higher than for successive drops of venous blood. For example, the average percentage coefficient of variation observed measuring platelets in venous blood was 4.6–4.8 percent, whereas for fingerprick blood, it was 19 percent – a significantly larger difference. Higher levels of variation were also observed for lymphocyte counts, granulocyte counts, and hemoglobin measurements (2). "In one donor, the hemoglobin concentration could change by 2 g/dL in just two drops of blood. This suggests caution should be exercised by groups developing tests to analyze tiny drops of blood, and to clinicians interpreting the results from such tests," says Meaghan Bond, first author of the study.

Complete blood count is one of the most common lab tests performed worldwide, and with increasing demand for point-of-care tests that use small amounts of blood, these findings have wide implications for the clinical community, adds Bond. However the issue isn't necessarily with the point-of-care technologies themselves, but could be down to the fact that samples contain extracellular fluid as well as blood, which may skew results.

But the news isn't all bad for researchers and startups hoping to harness fingerprick technology. Qualitative tests that provide a 'yes' or 'no' answer are less likely to be affected in cases where there is a high enough concentration of the studied analyte present in the blood. The authors also provide three recommendations for researchers working with fingerprick blood samples:

- Accept the inaccuracy of fingerprick blood as a trade-off for easy blood collection
- Collect, read, and average multiple drops (the study data suggests 80 µL is required) of blood to improve accuracy (though this requires more cost and time)
- When high accuracy is required, collect and analyze venous blood.

The team now plans to continue its investigation by testing the drop-by-drop variation of non-cellular blood components, such as glucose. *RM*

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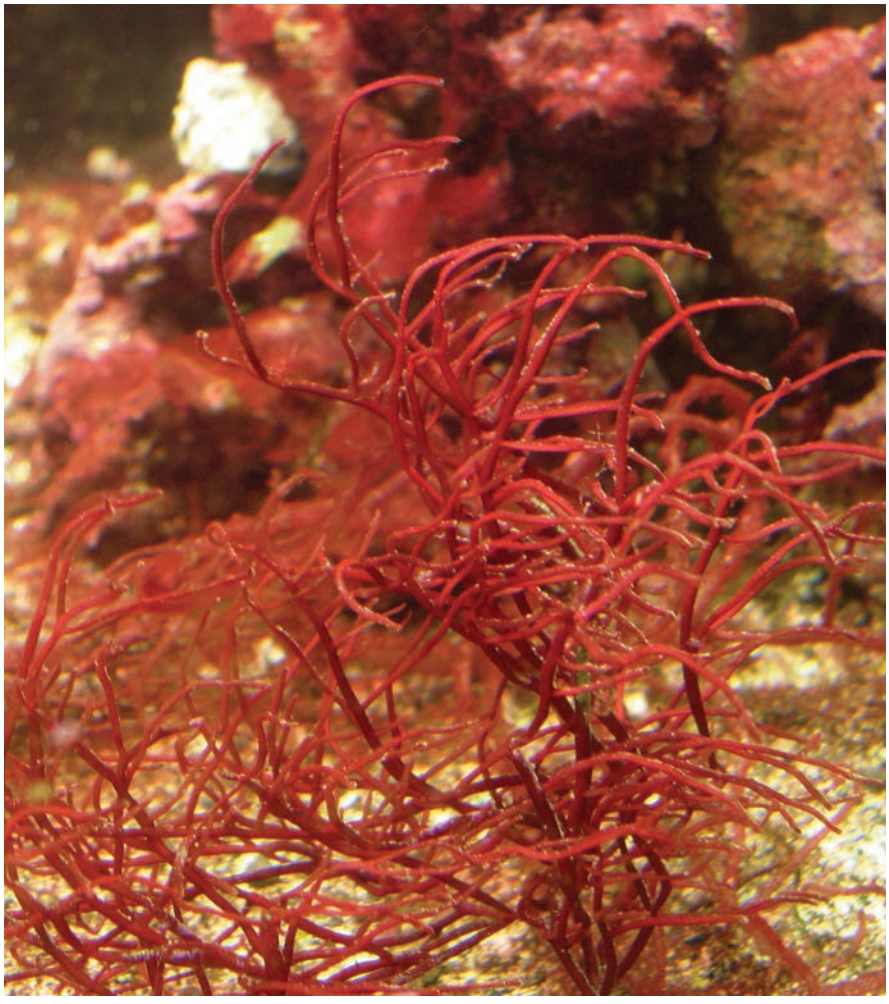
Under the Sea

Shortages of Gelidium are affecting the global supply of agar, driving up prices and causing decreased availability



Newly enforced trade restrictions on Gelidium seaweed, harvested predominantly in Morocco, are causing a shortage of an essential microbiology ingredient: agar. According to a recent report in Nature (1), with prices at an all-time high of US$35–45 per kilo, almost triple the normal price, this has obvious implications for researchers who rely on agar for microbial culture. Companies including Thermo Fisher are scaling back on the range of agar products they offer, says the report, and both suppliers and microbiologists could soon start to feel the pinch.

The trade restrictions were established in 2010 but only recently enforced, among concerns around overharvesting of the algae. Gelidium and similar types of red algae grow in naturally-occurring seaweed beds, and are harvested by divers or along the shore, making it impossible to simply increase production in line with demand. Pedro Sanchez, deputy managing director of the Spanish company Industrias Roko that produces around 40 percent of the global supply of agar, says, "It's not cultivated, and it's not possible to cultivate – although we've wasted a lot of money trying to do it in the past. We are forced to reduce our production according to raw material availability, and we estimate the reduction affecting bacteriological grade agar is around 25 percent."

Both trade restrictions and industry cutbacks have obvious repercussions for the microbiologists at the end of the supply chain. "We haven't been affected as yet, but if our agar supply does run low, we will not be able to carry out certain experiments," says Adam Roberts, senior lecturer in Molecular Microbiology, University College London, UK. "For example, when analyzing environmental isolates for antibiotic production as part of our Swab and Send project, we need to supplement the rich brain heart infusion agar with extra raw agar to achieve 4 percent agar. We couldn't carry out this project without the extra raw agar," he adds.

And if the shortage does worsen? "It would be a bit of a disaster really. In research laboratories it would hinder the progress of projects in multiple fields of research, and it wouldn't just affect microbiology – the more worrying outcome would be if clinical diagnostics labs ran short. This could impact patient treatments and potentially lead to treatment failure, for example, if an antibiogram cannot be performed", he explains.

As there is currently no suitable alternative to agar for culturing microbes, continuation of the shortage could have serious repercussions for microbiology. "If somebody came up with a suitable alternative they would be very rich very quickly" Roberts adds. *RM*

Reference

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Screening Out Controversy...

A new screening method for clinically significant prostate cancer may provide more precision and reduce the risk of overtreatment

To test or not to test? The controversy over prostate-specific antigen (PSA) screening is well known, with proponents of the test touting its potential to save lives and adversaries highlighting its risks (1). But this debate centers on the PSA test itself, rather than on prostate cancer screening – it's simply that, for many years, no better or simpler test was available. Now, researchers from Sweden's Karolinska Institutet may have an alternative to offer: the Stockholm 3 (STHLM3) model, a blood test designed to not only detect prostate cancer, but distinguish between low- and high-risk forms to prevent harmful overtreatment.

"PSA cannot distinguish between aggressive and benign cancer," says Henrik Grönberg, the study's principal investigator. "This leads to men who don't have cancer, or who have a form that doesn't need treating, going through an unnecessary, painful, and sometimes dangerous course of treatment. On top of this, PSA misses many aggressive cancers. Therefore, a more precise test than PSA is needed." The STHLM3 model, which analyzes a combination of six protein markers, over 200 genetic markers, and clinical variables, is intended to provide a comprehensive risk estimate for aggressive cancers. In a prospective, population-based, paired, screen-positive, diagnostic study of men aged 50 to 69, he and his colleagues compared the performances of PSA tests and the STHLM3 model in detecting clinically significant prostate cancers (2). The infographic summarizes their key findings.

"Based on the STHLM3 trial with 58,818 men [including the training cohort], we have shown that the STHLM3 test discovers aggressive cancer earlier than the PSA test and reduces the number of false positive tests and unnecessary biopsies," says Grönberg. "There has been great interest in the STHLM3 test both in the scientific community and among general practitioners." In Sweden, the new test will be available in clinical care from March onward, and the Swedish National Board of Health and Welfare will evaluate a national screening program later this year. Given the uncertainties that surround current screening for prostate cancer, practitioners may want to keep an eye on STHLM3! *MS*

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Key numbers

6 ..... Protein markers tested by STHLM3.

232 ..... Single nucleotide polymorphisms (SNPs) tested by STHLM3.

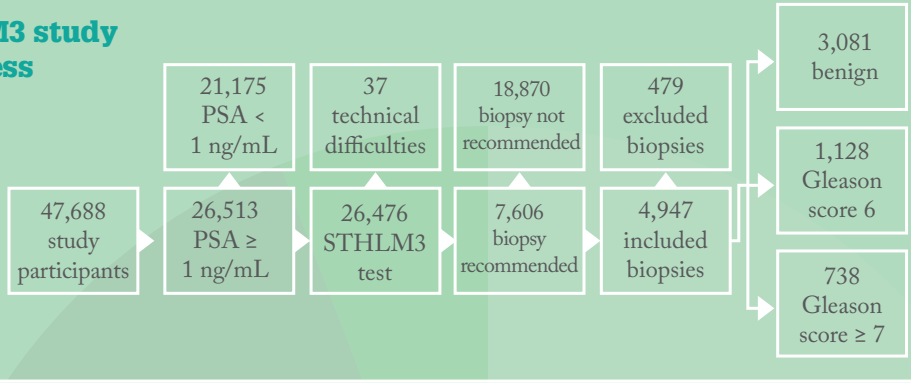
32 ..... The percent decrease in biopsies using the STHLM3 model.

44 ..... The percent decrease in benign biopsies using the STHLM3 model.

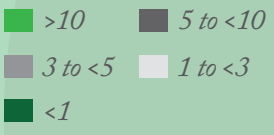
7 ..... The Gleason score of a clinically significant prostate cancer.

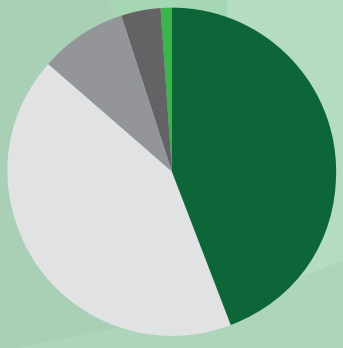
670,000 ... Men diagnosed with prostate cancer each year.

STHLM3 study progress

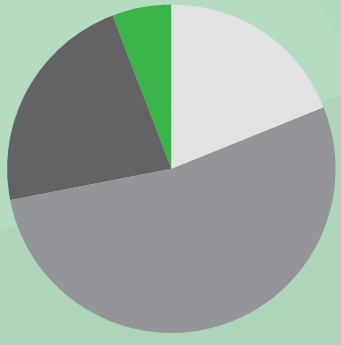


PSA scores of patients at inclusion





PSA scores of study participants at inclusion



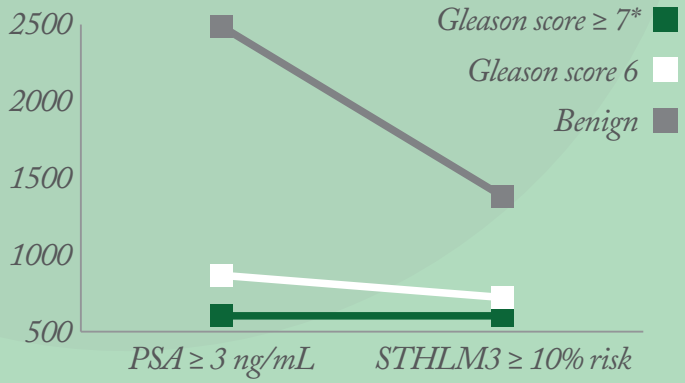
PSA scores at inclusion of participants who were ultimately biopsied

Number and outcome of biopsies

PSA ≥ 3 ng/mL
3,958

STHLM3 ≥ 10% risk
2,706

Total number of biopsies

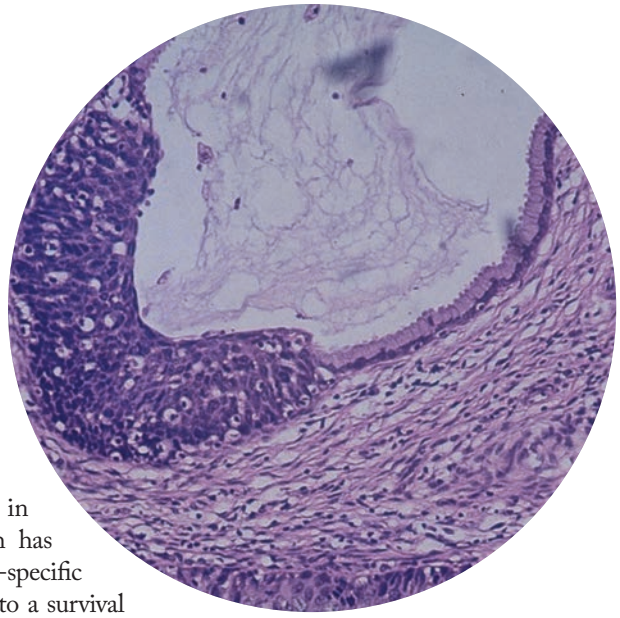


Biopsy results

*By Design

...And Challenging the Campaigns

As more and more emerging study data fails to definitively support cancer screening, is it time for a study design overhaul?



Cancer screening campaigns are everywhere. And despite disagreement within the medical community around effectiveness, the message most often delivered to patients is that screening allows them to catch cancer early, and increases their chance of survival. But does it?

With more studies emerging that are calling this into question, or can't provide a definitive answer, more patients are thinking twice about certain types of screening (1). And now, an article published in the BMJ (2) has further challenged preconceptions and thrown down the gauntlet for researchers, arguing that the way cancer screening is evaluated needs to change.

The crux of the argument is this: judging screening programs on their ability to reduce death from cancer is simply not enough, and assessing disease-specific mortality rather than overall mortality, fails to provide the entire picture. How many patients die of treatment complications (possibly after being diagnosed with a slow-growing cancer that wouldn't have caused death)? How many commit suicide? How many die of an expected downstream effect of screening? The studies being conducted now, argue the study authors, simply aren't powerful enough to answer these questions. And if you analyze what existing studies are able to tell us, the numbers aren't promising: a systematic review of cancer screening meta-analyses found that three out of 10 showed reductions in disease-specific mortality, but none showed overall mortality reductions (3). And ultimately, the authors state, overall quantity (and quality) of life is of most importance to patients.

"The big assumption in cancer screening research has been that lowering disease-specific mortality will translate into a survival benefit. But that's never been shown explicitly," says article co-author Vinay Prasad; "It is possible, and in some cases maybe even plausible, that avoiding death from one cancer may be offset by slight increases in treatment-related mortality, or mortality from off-target effects."

And it's not just the studies that need to improve, its patient education too. In one survey, 68 percent of women believed that breast screening would lower their risk of getting breast cancer, 62 percent thought it would at least halve the rate of breast cancer, and 75 percent believed that 10 years of regular screening would prevent 10 breast cancer deaths per 1,000 women (4). The real numbers are of course nowhere near as positive, with a 2013 Cochrane review concluding that, for every 2,000 women screened over 10 years, one woman will avoid dying of breast cancer, and 10 healthy women will undergo unnecessary treatment (5).

So what can be done to effect change? The BMJ article's authors suggest larger trials with the capacity to assess overall mortality, starting with participants in only the highest risk groups for the cancer in question. Although this is likely to be expensive, Prasad argues that "the amount of money and investment that western civilization is spending on cancer screening currently is orders of magnitude more than the cost it would be to really test these screening tests in very robust studies, for the endpoints that really matter to patients."

Prasad and his colleagues also recommend that patients should be given all the information they need to make well-informed decisions on screening; and they conclude with a call to action: "We call for higher standards of evidence, not to satisfy an esoteric standard, but to enable rational, shared decision making between doctors and patients." *RM*

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Predictive Protein

Citrullinated tenascin-C may offer the ability to detect incipient rheumatoid arthritis in a subset of patients as much as 16 years before the onset of disease

The word "arthritis" often conjures up mental images of people in the later stages of life – people who may have limited movement, need assistance with daily living, or claim to forecast the weather by the feelings in their joints. But new research shows that arthritis, even in its non-juvenile forms, may begin affecting the body long before it makes its presence known. A recently characterized marker reveals that predictive and diagnostic factors for rheumatoid arthritis may arise a decade and a half before clinical signs of the condition actually emerge.

Anticitrullinated protein antibodies (ACPAs) are well-known markers for diagnosing rheumatoid arthritis (1), and the cyclic-citrullinated peptide (CCP) assays used to capture them are designed for high sensitivity and specificity. What they don't do well is distinguish between subsets of ACPA-positive patients, meaning that they don't allow doctors to examine the mechanisms of a patient's disease or discern which treatment approaches might be most successful. Although ACPA assays can detect at least 20 different molecules, diagnostic tests for individual citrullinated proteins usually have low sensitivity. But a research team from the Kennedy Institute of Rheumatology at Oxford University have recently identified another peptide, citrullinated tenascin-C (cTNC), for which antibody testing has been shown to identify approximately 50 percent of rheumatoid arthritis cases with 98 percent diagnostic accuracy (2).

Anja Schwenzer, lead author on the study, says, "We knew that the protein tenascin-C could be found at high levels in the inflamed joints of people with rheumatoid arthritis. We decided to see if it could be citrullinated and, if so, whether it was a target for the autoantibodies that attack the body in rheumatoid arthritis." The authors analyzed blood samples from more than 2,000 arthritis patients from the United States, the United Kingdom, Sweden and southern Europe. "Around half of them have antibodies against cTNC," says Schwenzer, "including some patients who were not identified by the CCP test." The study had an even more exciting outcome; one in five people who went on to develop rheumatoid arthritis had antibodies against cTNC well in advance of clinical signs – on average seven years before the disease became evident, and as much as 16 years in advance.

"People testing positive for these kinds of antibodies could be monitored more closely for symptoms of rheumatoid arthritis and could therefore be diagnosed much earlier," says Schwenzer of the news. "That will allow doctors to start with the right treatment much earlier, making it more effective and also making it much easier to control the disease." But first, she and her group would like to find out whether testing for these antibodies would help identify patients at risk of developing a more severe form of the disease. They'd also like to know whether certain patient groups – like smokers or those with gum disease – are more likely to exhibit elevated cTNC antibody levels. Factors like these may make cTNC a useful predictor of disease onset and perhaps even help guide doctors to more appropriate treatments for their patients. *MS*

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The Smaller Picture

Research group attempts to establish the evolutionary behavior of cancer cells, with interesting results…

It is often thought that cancer cells follow a Darwinian model of evolution – but a recent study challenges this view. Lead study author (1) Chung-I Wu and colleagues decided to take a look at the smaller picture: studying just one small tumor (slightly smaller in size than a ping-pong ball) derived from a hepatocellular carcinoma. They found that it contained over 100 million mutations within coding regions of its DNA – a much higher mutation rate than they expected. We spoke with Wu about the implications of this discovery…

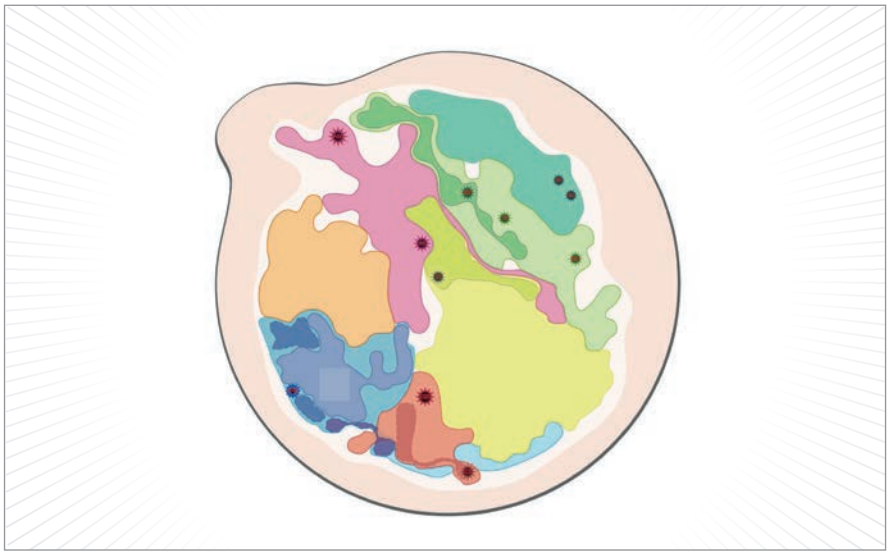

Figure 1. Map of a tumor. Each color denotes a different clone. Each star indicates a "singleton" clone that is unique among the samples. Note the outward expansion from the center and the overlapping clones.

Why study just one tumor?

Every population geneticist's first question about any population is "how much genetic diversity is there?" and "how is the diversity distributed?" The amount and distribution of diversity informs us about the demographic history of the population (how big it is, how much it has been growing/declining, how much subdivision there is, and so on), the influence of natural selection, and the possible evolutionary trajectory of the population in the future.

The cancer cells within just one tumor represent a very interesting population, and we want to study the evolution of each case of cancer with the same rigor as if we were studying one single species. This is in sharp contrast to the prevailing strategy in cancer biology, which advocates studying large numbers of cases with minimal depth for each case. We plan to study no more than 10 cases in the years to come, with very extensive sampling within and between tumors of the same patient.

What did this first study tell you?

First, the diversity within a tumor accumulates in a non-Darwinian mode. In cancer biology, it is almost always assumed that Darwinian selection dominates cellular evolution. This thinking echoes evolutionary biology 50 years ago. But in 1968, the advent of the neutral theory of molecular evolution changed this view drastically. Much of the evolution within natural populations is perceived as neutral, and not driven by Darwinian selection. Our key finding is that non-Darwinian evolution appears to be as dominant at the cellular level as it is outdoors in natural populations.

Second, the level of genetic diversity is orders of magnitude higher under non-Darwinian than under Darwinian evolution. This is intuitively understandable when we look at cancer cells, as Darwinian selection would throw out bad mutations and keep a very small number of the best mutations, whereas almost every mutation is kept under the non-Darwinian mode (Figure 1).

What could your findings mean for oncology?

The near certainty of drug resistance is the inevitable consequence of non-Darwinian evolution when there are hundreds of millions of coding region mutations. High genetic diversity is seen even when the tumors are microscopic. The medical implication is this: if complete eradication is untenable, what would be the rational strategy to control and contain the growth of tumors? There are many options, but acceptance of the pre-existing resistance is necessary before such options can be evaluated. An immediate question to look into is finding the best treatment strategy right after surgery, when the tumors remaining are microscopic, but also highly genetically diverse.

Reference

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Rooting out Resistance

New software for analyzing genetic data could bring simple antibiotic resistance information to your tablet or laptop

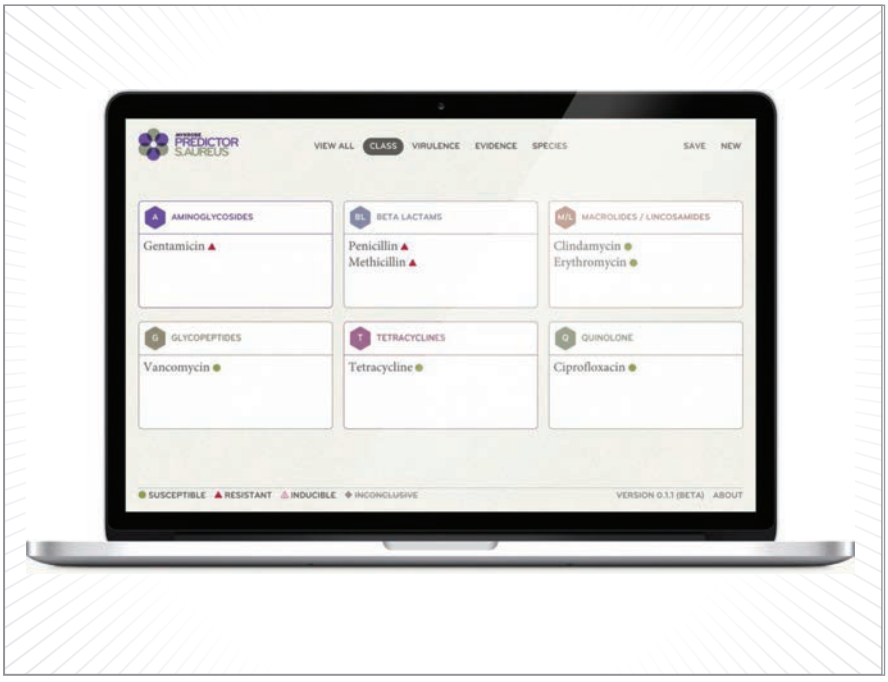

Figure 1. A screenshot from the Mykrobe Predictor for *Staphylococcus aureus*, showing the easily interpreted results screen.

Antimicrobial resistance is a huge and ever-growing problem, and it's a known fact that, if doctors and researchers want to stay one step ahead of the superbugs, new antibiotics and diagnostic tools are needed. One such potential weapon is Mykrobe Predictor, a computer program that can provide drug resistance information in a few minutes using gene sequencing data from a bacterial infection, according to its developers.

Traditionally, drug susceptibility testing is used to find out which drugs an infection is resistant to, and involves introducing different antibiotics to a culture of the bacteria, but this process can take days, or potentially even longer for slow-growing pathogens like tuberculosis (TB). The Mykrobe program looks directly at the bacterial DNA and quickly analyzes it, presenting the results in a format that does not require specialist interpretation (see Figure 1).

A retrospective study of over 4,500 patients found the prediction software accurately detected antibiotic resistance in *Staphylococcus aureus* and TB – detecting resistance to five antibiotics in over 99 percent of *S. aureus* cases, comparable to the performance of drug sensitivity testing. For TB, sensitivity was lower (82.6 percent), presumably because the genetic basis of resistance in TB is not as well understood – but the test matched the performance of current DNA tests, and returned a result in just three minutes; weeks faster than a drug susceptibility test can. According to the study authors, the software can also identify mixtures of drug-resistant and drug-susceptible microbes.

But the technology isn't just aimed at the diagnosis and treatment of individual patients. "The key is this," says senior author of the associated paper (1), Zamin Iqbal, "Current mix-bug-and-drug methods are all about the patient. Get a result for the patient, treat them, then throw the data in the bin. Maybe not literally, but currently the information that patient X had an infection that was resistant to A, B, or C drugs is useless to anyone else. But using genome sequencing, we can get the resistance information for the patient and detailed information on the ancestry of the sample – i.e., which strain it is. We can then share this digitized information globally, because bacterial DNA contains no patient information, and contribute to global tracking of bacterial strains, and of drug resistance."

The software is now being evaluated for clinical use in three UK hospitals, with the aim of testing the technology on real clinical samples, resolving any issues that arise for the microbiologists using Mykrobe, and collecting more data on rare mutations. The complex data that arises from whole genome sequencing is a major barrier to its adoption by the National Health Service, adds Iqbal. The developers hope that programs like Mykrobe could help to overcome this problem and provide fast genetic information to better treat patients, and better understand antibiotic resistance. *RM*

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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 editor at fedra.pavlou@texerepublishing.com

What Will Red Tape Mean for LDTs?

New regulations in the US set to change the way LDTs are developed – and could stifle innovation

By James Nichols, medical director, Clinical Chemistry and Point-of-Care Testing; professor of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA.

Let's begin by defining laboratory-developed tests (LDTs). These are tests created because a commercial test is not currently available. Examples include urine testing for drugs of abuse by mass spectrometry, serum catecholamine testing by high performance liquid chromatography, and blood volatiles analysis by gas chromatography. Modified US Food and Drug Administration (FDA) approved tests to fill an unmet clinical need are also classed as LDTs, for example, body fluid chemistries (amylase in peritoneal fluid) are LDTs because the test's FDA approved sample, serum/plasma, is modified to analyze a different fluid (peritoneal fluid, in this case). Also, many tests performed by clinical laboratories are LDTs, including infectious disease testing, chromatography, molecular diagnostics, and even point-of-care testing when a device is used outside manufacturer claims or off-label from the package insert. The list is seemingly endless!

Because these tests are developed in the lab, does not mean that they are immune to the scrutiny of the regulators, though, and changes to the regulatory process in the US are afoot, which some argue, could stifle innovation and even access to much needed tests.

The 1976 medical device amendments to the Federal Food, Drug, and Cosmetic Act, gives the FDA the authority to regulate all laboratory tests, regardless of whether they are commercially distributed or developed by a laboratory. According to the Act, the FDA must ensure that *in vitro* diagnostic devices are safe, effective, and perform as claimed for their intended use, so patients are not harmed. So far, the FDA has used enforcement discretion towards LDTs by not enforcing some or all applicable laws and regulations. LDTs are also subject to quality regulations under the US Centers for Medicare and Medicaid Services (CMS) Clinical Laboratory Improvement Amendments of 1988 (CLIA) as high complexity tests.

Recently, however, the FDA has announced that it will begin regulating LDTs much like commercially-marketed diagnostic tests (1,2). How will this work? Laboratories creating LDTs will need to submit the test performance claims to independent premarket review. They must also assure the test provides clinically meaningful results (clinical validation), adhere to quality system requirements in production, and be subject to post-market reporting and surveillance of test incidents. This won't happen immediately, though. The FDA proposes to start with those tests presenting the greatest risk to public safety while continuing enforcement discretion for some low risk LDTs.

This change in FDA guidance poses a number of operational and practical issues for clinical laboratories. Having two regulatory oversight agencies (FDA and CMS) with overlap in their requirements will be confusing. Manufacturers have regulatory affairs departments devoted to handling the requirements of test approval. However, the rigorous processes of FDA submission, quality system regulations and

medical device reporting are entirely new concepts for clinical laboratories and they will require additional staff resources and training. Laboratories will face additional expenses to conduct the studies required for clinical utility of the test as well as filing fees for review and ongoing device taxes imposed once the LDTs are approved.

Seeking FDA approval will also impose delays and disruption in clinical care. And, we'll need longer lead times to conduct the studies required for submission. Indeed, some labs may even be unwilling to submit their LDTs for review because of the added expense and resources required to meet this change in FDA guidance. This will lead to possible discontinuation of some currently available LDTs and risk limiting patient access to tests. And, there is uncertainty over how FDA and CMS will interact once the regulations are imposed, and how private accreditation organizations will need to change their inspection process.

The FDA proposes initial enforcement for high-risk tests with a phase-in of the regulations over several years. How risk will be decided for specific tests and whether labs will have to stop conducting LDTs while going through FDA review is not clear at this time. Modifications of FDA-approved tests and whether labs will need to submit them as a new LDT is also not clear. There are currently many unanswered questions.

I believe that these proposed FDA changes, while intended to enhance LDT quality, may actually have the opposite outcome. Increased costs could prevent labs from developing new LDTs and stifle future development. Increased regulations could lead to removal of currently offered LDTs from the market. Limited FDA staff and resources could delay review of pending submissions given the flood of new submissions from clinical laboratories. Dual systems of oversight from FDA and CMS with overlapping requirements and different viewpoints could confuse the market and further discourage new test development. Most importantly, the increased oversight could interfere with current physician-laboratory director relationship that fosters LDT development and professional test result interpretations within an institution. The future impact of this change in regulatory approach feels quite overwhelming.

Recent discussion at the annual 2015 AACC meeting in Atlanta were promising, however. A working group of the FDA and CMS has been formed to streamline the LDT review process and reduce agency overlap. In addition, the FDA announced its flexibility and openness to publicly discussing these issues and working through the challenges of the proposed changes with the clinical laboratories and diagnostic manufacturers. I have no doubt that the entire clinical laboratory industry will look forward to these discussions over the next several months and it will certainly be interesting to see what the overall impact these changes will have on laboratories and, importantly, on patient care.

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Why POCT?

Does point-of-care testing have a true positive effect on patient outcomes, or is it simply a perceived benefit?

By T. Scott Isbell, assistant professor of Pathology, medical director of Clinical Chemistry and Point of Care Testing, Saint Louis University School of Medicine, Missouri, USA.

Point-of-care testing (POCT) is a growing trend. Examples of frequent inpatient POCT measurements include whole blood troponin for assessing myocardial injury, creatinine with estimation of glomerular filtration rate for assessing renal function, prothrombin time (PT) with INR calculation to guide thrombolytic therapy in stroke protocols, and the ubiquitous measuring of whole blood glucose for managing a patient's glycemic state.

Today, you will see POCT throughout a hospital with handheld devices or small benchtop analyzers in the emergency department, intensive care setting, general medical wards, labor and delivery, and imaging centers. And market analyses seem to reflect this trend of moving testing away from central laboratories and to the patient's bedside. According to a MarketsandMarkets 2014 report, for example, the global point-of-care diagnostics market is projected to hit US$27.5 billion by 2018 – indicating a clear demand for this type of testing. The market research firm says the growth is

due to rising prevalence of chronic and infectious diseases, increasing use of home-based POCT devices, technological advancements, and the decreasing number of technologists in the central laboratory, to name but a few. In my institution, some administrators have also considered POCT as a way to lighten the workload of a resource-strained central laboratory.

This demand is reflected locally with plentiful requests from my clinical medicine colleagues. The ability to perform a rapid biochemical analysis at the bedside, with results sometimes available in seconds rather than hours, is very appealing to the physician. The perceived benefit of POCT is that faster results lead to faster changes in management – meaning that patients will have better outcomes. While logical, there's no proof yet that this is actually true. It is, however, a testable hypothesis, with well-designed, outcomes-based research studies that evaluate the effectiveness or efficacy of such testing.

However, outcomes research on POCT (or any laboratory tests, for that matter) is difficult because the tests don't have a direct impact on the patient; instead, they require action by clinicians. I think Petrie Rainey, a professor of laboratory medicine at the University of Washington, USA, summarized this well in his 1998 editorial for Clinical Chemistry (1). He wrote, "…the effect of a test result is always filtered through the change in medical management it engenders." James Nichols *et al.*, in a 2000 study evaluating patient wait times after POCT tests in an interventional radiology and cardiology setting, very nicely confirmed Rainey's observation (2). Nichols' group demonstrated a decrease in wait time only after optimizing workflows around the POCT results. Fast-forward 15 years and this observation still likely explains why so many studies continually fail to show positive outcomes associated with POCT.

A hotbed of POCT outcomes research is the hospital emergency department (ED). Here, POCT has been proposed as the solution to overcrowding (3). The specific aims of POCT in the ED are to increase timely discharge, shorten length of stay, increase patient throughput, and reduce time to treatment. This begs the question – does POCT accomplish the aforementioned aims? Numerous randomized controlled trials have been conducted, for example, comparing POCT with central laboratory measurement of cardiac biomarkers. Roland Bingisser *et al.* summarized their group's findings in a 2012 American Journal of Emergency Medicine article (4). Based on my understanding of the article, I think that collectively these randomized controlled trials indicate that point-of-care cardiac biomarker testing has little to no impact on length of stay, which raises questions about the utility of performing POCT when a central laboratory measurement may suffice. It is important to note that managing POCT requires considerable resources and time to ensure quality testing is being performed.

Don't get me wrong – I do think there can be tremendous benefit to POCT if results are acted upon in a timely manner. The big question is: will we abandon POCT if well-designed studies (incorporating optimized workflows) reproducibly fail to demonstrate a positive effect, or will we continue to perform POC testing under the guise of a perceived benefit? I look forward to seeing the data!

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Behind the Curve

Education and new tools critical to solving the slow adoption of updated reference assembly data

By Valerie Schneider on behalf of the Genome Reference Consortium (GRC)

The Human Genome Project (HGP), in producing the human reference genome assembly, generated a pivotal resource that promised to transform our understanding of human biology and change the future of medicine. The availability of the reference assembly and the ensuing research have transformed the way we see ourselves, the way we diagnose disease, and in some cases, the way we treat patients. Success and reliability for each of these things depends upon the quality and completeness of the genomic data.

Errors, mis-assemblies and incomplete or missing data in the human reference assembly all have the potential to undermine downstream analyses – including diagnostic testing resources. The Genome Reference Consortium (GRC*) was established after the conclusion

of the HGP to improve the assembly, ensuring it continues to represent our latest understanding of human genomic biology and serve as the best possible substrate for efforts to advance human health. Though it seems obvious that outdated or incorrect data can negatively affect analyses, researchers and clinical testing labs are generally slow to transition to new assembly versions. In turn, assembly improvements are slow to reach the public.

The failure to transition to a new version largely results from two factors: ignorance of existing assembly problems or new assembly improvements, and limited resources – for data migration, interpretation of migrated data, or use of new assembly model features. Clinical labs are further hampered by the lack of robust validation sets for confirming tests on new assembly data. As long as the cost of transitioning data is perceived to be greater than the cost of using outdated and incorrect data, there will be resistance to the adoption of updated assemblies.

To changing this perception, we need not only education and resource development, but also the combined efforts of the GRC and tool developers working in basic and clinical settings. Therefore, GRC workshops at the 2015 meetings of the American Society for Human Genetics and the Association of Molecular Pathologists explored the human reference assembly, highlighted recent changes, and discussed their clinical and diagnostic implications.

The latest version of the human genome reference assembly, GRCh38, reflects nearly four years of curatorial efforts by the GRC. These include the resolution of more than 40 issues reported by clinical labs and known to affect development or interpretation of genetic tests. For example, an inversion error in prior assembly versions precluded a reference representation for the PTPRQ gene (which, when mutated, is associated with deafness) and has now been corrected. In other cases, the addition of new chromosomal sequences to GRCh38 have added entire gene representations that were missing from previous assembly versions, such as KCNJ18, in which some variants result in thyrotoxic periodic paralysis. Improved reference representation of these and other disease-associated genes in GRCh38 should not only permit the development of more complete genetic testing panels but also improve interpretation of test results.

Tools like the NCBI Genome Remapping Service, UCSC LiftOver, and Ensembl remapping API facilitate adoption of new assemblies by transforming data based on one assembly version to another. However, not all data will map from one assembly to another and some will map to multiple locations, complicating analyses. Additionally, in certain situations data may map differently depending on the remapping resource used. Having a greater appreciation for the reference assembly and the changes it has undergone makes it easier to understand remapping discrepancies and their possible implications for data interpretation.

Assembly improvements also include additional allelic sequences that exist outside the chromosome coordinate space. Alternate loci scaffolds provide representation for genomic regions at which a single chromosomal sequence representation is insufficient to capture population genomic diversity. GRCh38 provides more than 30 different representations of A and B haplotypes in the KIR region, where genetic variation is associated with susceptibility to autoimmune disorders and possibly HIV infection. Likewise, the new assembly contains a growing representation of genomic variants at the CYP2D6 locus, a gene that plays a critical role in the metabolism of many drugs.

Patches represent another class of extrachromosomal sequences and provide fast access to assembly corrections and new variants between the infrequent major coordinate-changing releases. Additional CYP2D6 variants have been added since the initial GRCh38 release as novel patches, while fix patches have further improved gene representation in the assembly. However, some labs and organizations do not yet recognize or include the more than 150 off-chromosome gene representations, precluding their use entirely in the development of new resources. Furthermore, many of the file formats and tools used for variation analysis have not yet evolved to use these additional sequences, which makes the barrier to their adoption even more difficult to overcome.

Timely adoption of assembly updates requires effective education about the reference assembly, tools to facilitate the transition of data between assemblies, and workflows that fully support all sequences in the reference genome assembly. More information about all these things is available from GRC workshops, as well as the GRC website (http://www.genomereference.org) and publications (1,2). The GRC welcomes feedback, especially from the clinical community, about their experience with prior and current reference assembly versions.

**The GRC is a collaboration between The Wellcome Trust Sanger Institute and the European Bioinformatics Institute (Hinxton, UK), The McDonnell Genome Institute at Washington University (St. Louis, USA), and The National Center for Biotechnology Information (Bethesda, USA).*
Valerie Schneider leads the National Center for Biotechnology Information (NCBI) team for the Genome Reference Consortium (GRC), the group responsible for updating the reference genome assemblies for several organisms, including human and zebrafish, in Bethesda, Maryland, USA.

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An Award-Winning Career

Voted Number One on The Pathologist Power List, Manuel Sobrinho-Simões, talks profession, politics, education, innovation, and the ups and the downs of a career that has inspired generations of pathologists.

By Fedra Pavlou

Last year we asked our readers who they believed were the most influential figures in pathology and laboratory medicine, and in November, after open nominations and judging by an independent, multidisciplinary panel, we celebrated the top 100 (1). And the number 1 spot? It was awarded to Manuel Sobrinho-Simões, founder and director of globally-renowned IPATIMUP (the Institute of Molecular Pathology and Immunology of the University of Porto), a man who was described by nominators as someone who "represents the perfect combination of scientific intelligence and nobility," is "an educator par excellence," and whose "contributions to the clinical diagnosis of thyroid cancer have been outstanding." Here we speak with the pathologist who has taught and inspired thousands of students and made ground breaking contributions to the field of thyroid oncology. Manuel Sobrinho-Simões speaks with The Pathologist about his diverse and interesting career, his focus on education, his fears surrounding overuse of molecular and digital pathology. And he tells us what he still has to do…

Let's start at the beginning… Why pathology?

My great-grandfathers, grandfathers, father and uncle were all medical doctors, so I never considered doing anything else. When I began studying medicine, I found myself drawn to pathology because I realized quite quickly that I didn't want to be a clinician – I wasn't interested in interacting directly with

patients; I wanted to diagnose disease on an objective basis. Pathology was the best possible solution. I was very impressed by my Professor of Pathology (Daniel Serrão) and my interest lay quite firmly in human pathology.

"The political situation was beginning to destabilize. A revolution was on the horizon and on April 25, 1974, 50 years of dictatorship was ended."

How did the political situation in Portugal at the time affect your studies?

We had a strong political regime, which made it very difficult for students to undertake a PhD overseas – the government were convinced that you wouldn't come back. So as students, it was generally accepted that we had to do our PhDs in our home country. This was a challenge for me, because I wanted to study human pathology – a subject that was only considered to be observational and not good science. I had some convincing to do! At the same time, the political situation was beginning to destabilize. A revolution was on the horizon and on April 25, 1974, 50 years of dictatorship was ended by army rebels on a day that was known as the Carnation Revolution. It was a very special time in Portugal; in a way, it was a little overboard – the freedom went to people's heads and there was a lot of spending, a lot of excess. I was a slight leftist, so those changes weren't very easy for me to deal with. Still, I have very good memories. Before the revolution, there were not many medical students – I would say between 60 and 80 per year. After 25 April, student intake increased to more than 1,000 per year. The situation reverted partially and, today, my medical faculty enrolls about 300 students per year.

I was able to convince the director of my department that by studying human pathology, I could solve a lot of problems and confirm diagnoses using a new, high-tech method – electron microscopy. That assumption was, I later discovered, a naïve one, but it allowed me to successfully gain approval to undertake my chosen PhD. I became the very first pathologist in my home city of Porto to do a PhD in observational human pathology.

Why did you choose to complete your postdoc in Norway?

I had a keen interest in electron microscopy. Jan Vincents Johannessen was an exceptional electron microscopist based at the Norsk Hydro's Institute for Cancer Research in Oslo, so that was a huge draw, and in 1979–1980 I worked alongside him studying thyroid cancer and electron microscopy.

I then returned to Portugal to continue my career. By 1988, I became a full professor of pathology at the University of Porto. Through the course of my career, and even now, I have worked overseas for short periods. I always combined my profession with research, and I have always tried to teach. Why? Because I wanted to be as good a professional pathologist and as good a researcher as possible; not because I wanted to do research *per se*, but because I wanted to be a better teacher.

In 1989, you founded the Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP). How did you go about securing funding?

That was difficult. By that time, we had realized that electron microscopy was not the magic bullet I had hoped it would be. We needed to prove to our government that we could use sophisticated techniques to do first-class research. Those sophisticated techniques were immunohistochemistry (IHC) and molecular pathology. Not only could we use

them to improve our research, but with their help, we could improve patient care and diagnoses. People are talking a lot about translational medicine these days, but we were doing translational work from the very beginning!

I must confess that, at the time, pathology in Portugal was very prestigious because we had three very good schools for the subject. It was seen as an important discipline politically; some of the most brilliant medical students wanted to enter pathology. It's not the same now, but that's how it was then. I was not alone in convincing the government to part with funds – we had brilliant students, and between 50 and 75 percent of our team were faculty professors of the University of Porto who were volunteering their services for free. Which was fantastic; it was unique and it was lucky. We also had a brilliant Minister of Science who wanted to reinforce science and who was actually a little suspicious of the universities; he wanted to create solid research institutes. Luckily, at that time, the director of the University of Porto was a real visionary and he supported us too. We were able to convince the government that we could produce good science and that our science would have a direct, positive impact on patients. We had to demonstrate the value of our work from day one, and that was how we managed to secure all of the funds that we needed – 25 percent of which came from Portugal and the remaining 75 percent from the European Union – to build our institute. IPATIMUP was, and still is, a rarity in Europe, because of its focus on human pathology.

Everything fell into place and we quickly began to demonstrate how useful we were. It was a great success; the newspapers, TV and radio all loved us. In such a small country, having a big media presence was like advertising our work with a megaphone. We won the National Media Prize for Science in 1996. And for the first time ever, it was not awarded to an individual, but to a group – the "Professor Sobrinho-Simões group".

One thing that really helped us gain media attention was our communication with the public about health and disease – in particular, cancer. In Portugal, we have a low level of literacy. At the time of the revolution, literacy rates were similar to those of Sweden 120 years earlier! So it was not easy to communicate

scientific information to the public, but we managed to do it in such a way that we taught the country that cancer was a chronic disease, that it needed to be diagnosed early, that it was treatable, that it could be prevented, and that it was not a death sentence. Our constant presence in the public eye and our internationally competitive research secured our future.

Why did you focus research on thyroid and gastric cancers? And what are the institute's biggest achievements?

The prevalence of both of those cancers is very high in Portugal in comparison to other countries, but neither was widely researched at the time and we were able to become experts in both fields. From 2008 to date, the University of Porto has been one of the top five global institutes by number of citations in thyroid and gastric cancers and this is because of IPATIMUP's influence over the university hospitals and cancer institutes. In my opinion, this is our biggest collective achievement.

Another rewarding accomplishment for us was that we were able to create, together with the Cancer Hospital, the Porto Comprehensive Cancer Center – the only one in Portugal and one of a very small number in the Iberian Peninsula.

Scientifically, we have published many good papers. For example, we were the first to establish that the most frequently occurring thyroid and endocrine cancer in humans – papillary thyroid carcinoma – often has a *BRAF* mutation (2). Two years ago, we found another mutation in thyroid cancers; this time, in the promoter of the *TERT* gene (3). You could say we are responsible for finding two of the most frequent genetic alterations in thyroid carcinomas. We were also the first group to find a genetic germline alteration in a special type of cancer known as Hürthle cell tumor (4). Finally, because of our focus on translational research and human pathology, we were constantly collecting information on the relationship between genotype and phenotype, and in 2006 we published a paper redefining the existence of two major subtypes of thyroid papillary carcinoma, one of which was similar to follicular carcinoma (5). More recently, using high throughput technology and NGS, The Cancer Genome Atlas Research have managed to confirm our findings of 2006 (6). I now do more than 200 consultancy cases of rare thyroid cancer each year from around the world, and I do them for free. These cases

are great, because they are triggers for research.

In stomach cancer, two of my colleagues – Fatima Carneiro (also named on The Pathologist's 2015 Power List) and Raquel Seruca – are considered among the world's top experts in hereditary diffuse gastric cancer. They discovered the mechanism, the interaction between mutations and the microenvironment including the role of *H. pylori*, and more. They are asked to consult on cases of diffuse gastric cancer from around the world.

"The development of all sorts of sophisticated imaging techniques and the excessive belief in the importance of molecular alternatives is already leading to overdiagnosis."

It's very difficult to extrapolate information from animal models. For years we just focused on human molecular pathology, and that's what has allowed us to become leaders in our fields. Furthermore, most of our research achievements have been the result of multidisciplinary collaborations with global researchers.

In 2007, we joined forces with the University of Porto and two biomedical and life sciences research institutes to build a new institute that is linked with IPATIMUP – the i3S (Institute of Research and Innovation in Health). IPATIMUP is 4,000 m^2 and now with the Institute, we have an additional 14,000 m^2 containing about 600 researchers. This is in the middle of the university hospital, the cancer hospital and medical faculty campus, so we probably have one of the best health campuses in Europe. I'm very proud of that. It's a unique opportunity to translate our research into benefits for patients.

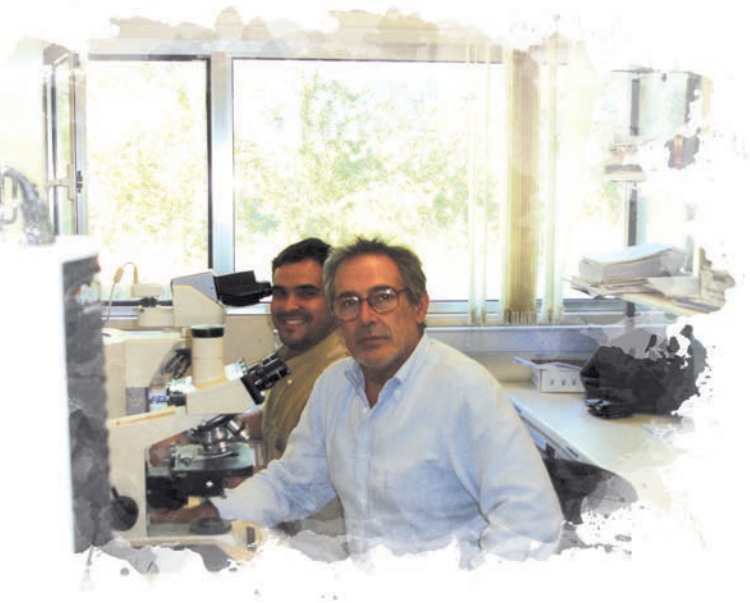
What have been the biggest evolutions in pathology since you began practicing?

After the introduction of immunohistochemistry there have been a lot of changes, mainly on two levels. Our ability to conduct genetic analysis on large numbers of samples at such high speeds and at relatively low costs, and our integration of digital technology.

You call molecular pathology a double-edged sword. Why?

Don't get me wrong, it is a fantastic field! But it's very difficult for classically trained pathologists to understand all of the new molecular techniques – and many of them are afraid. It's an intimidating prospect, so they aren't trying to use molecular pathology. And this is bad, because they need to. Conversely, some people think that molecular pathology is the answer to all of our diagnostic questions – and it's not. Though we're discovering more and more about the molecular nature of disease, the diagnosis of cancer is still based on morphology and topography. It has to be. So this is why I think of molecular pathology as a double-edged sword; because it holds a lot of promise for the future (if we can get pathologists to participate), but it should not be regarded as all-powerful.

I must stress the danger of overdiagnosis in cancer and precancerous diseases. The development of all sorts of sophisticated imaging techniques and the excessive belief in the importance of molecular alterations is already leading to overdiagnosis and overtreatment. I find it noteworthy that we have needed to create acronyms like VOMIT (victims of modern imaging technology) and the concept of IDLE lesions (indolent lesions of epithelial origin) for *in situ* "cancers" and extremely low-aggressive malignant neoplasms. In 2003, we had a meeting in Porto with Dillwyn Williams, Juan Rosai and Virginia LiVolsi, during which we established the so-called Porto proposal, which suggested the name papillary microtumor as a replacement for papillary microcarcinoma (of the thyroid).



I would urge caution with liquid biopsies too; specifically the way that the data is used from these tests. The study of primary tumors and their metastases needs to include a detailed description of the tissue heterogeneity, together with topography. Cancer is, by definition, invasive. It does not respect frontiers and therefore can only be diagnosed with morphological (and/or imaging) methods. A gene alone cannot diagnose cancer or highlight invasiveness and I think the same will continue to be true in the future. The over-evaluation of molecular genetics is dangerous in this context. On the other hand, if we don't integrate molecular and gross testing techniques, we will lose ground to other disciplines. Our approach to diagnosis must intelligently integrate both.

Further, with our better understanding of genetics and continuously improving technologies, we are increasingly able to access huge amounts of information. This does present a problem, though; it is difficult to reconcile all of this information with actual cases. We still have a gap. Bioinformatics is a weak point within our institute – we have a limited capacity to handle all of the information we can obtain, and I don't know how we are going to solve that problem. We were a little bit naïve in the beginning because we thought that looking at genes would solve everything – but that's not true. Many diseases don't depend on genes *per se*, they depend on epigenetics and environmental factors. We still don't have enough understanding of the epigenetic influence on disease.

And you're not a big fan of digital technology?

Personally, I am ambivalent. I don't even own a cell phone, so that proves to you that I am a traditionalist. That extends to my work as well – I prefer to use my microscope. Many people agree that they feel more secure behind the microscope, especially those that were trained classically. It's quicker and easier for us to make a diagnosis that way than by using a computer. My biggest concern with digital pathology ties in with the fact that, globally, we are suffering from a shortage of pathologists. With the support of digital technology, I'm afraid that we might have fewer pathology departments within hospitals, and more digital pathology supercenters around the world, in countries where it's cheap to run them. Digital pathology has the potential to contribute to the decline of our profession. And that would be a disaster!

Don't get me wrong, I do see benefits to using it. I believe it will solve many problems – in particular for remote laboratories that are unable to conduct certain tests or that don't have the resources. But digital tools should be controlled and used effectively. When testing DNA or RNA, for instance, it's very important to have control over the preanalytical phase, and that is lost when you outsource the analysis. I wouldn't use digital pathology for surgical specimens, either. I still believe that to study cancer specimens properly – because it's a disease that needs an integrated, multidisciplinary approach – we need to use traditional techniques. To do that, pathology needs to be within the hospital.

"Digital pathology has the potential to contribute to the decline in our profession. And that would be a disaster !"

You say you're concerned about losing pathology departments...

That's right. Subspecialization will drive the loss of pathology departments, in my opinion, where, for example, the gynecological pathologist is simply integrated into the gynecology department. I fear that we will lose our unity and our visibility as a result. Perhaps of even more concern, is the fact that this consequence would damage our chances of providing the best personalized medicine to our patients. In-house pathologists promoting the intelligent use of tissue and tumor banks will become increasingly more essential for the delivery of personalized medicine in oncology; and such banks should, I believe, belong to pathology departments.

In Porto, thankfully, we're currently not at risk of this because pathology is still viewed as the most important discipline in the medical curriculum. We are lucky. I currently teach pathology at the medical faculty of Porto, as does my colleague Fatima Carneiro, and our pathology courses are exceptionally

strong. But I see subspecialization causing problems in other countries and in the future.

We're also seeing public universities and hospitals losing pathologists to private labs. Why? Better salaries. This is also a big problem.

How can this be avoided?

Three things need to happen. One, we need to pay our pathologists more – the workload is high and the compensation does not reflect the complex and demanding nature of the profession. Our pathologists contribute so much and we should make sure they're happy. Two, we need to better reward research. And three, we need to be more visible as a profession.

Pathology is still a prestigious profession in Portugal. How can other countries follow suit?

Through the mobility of the pathologists trained in good overseas institutions who return to their home countries or circulate around the world. We train a lot of people from Portuguese-speaking countries like Brazil and Mozambique, as well as people from Spanish-speaking countries, and these people move around. Take another of the people named on the Power List as an example, Jorge Reis-Filho; he is a brilliant pathologist who trained with us and is now based at Memorial Sloan Kettering Cancer Center in the United States. We need pathologists to spread their influence around the world. Pathologists should be role models as professors in medical faculties and as clinically-oriented, physician-scientists in hospitals. This is why I have been so focused on the European School of Pathology. I started collaborating with the European Society of Pathology (ESP) as co-editor of their newsletter in 1980. After having chaired the Advisory Council from 1985–1989, I was Secretary from 1989–1997 and served as President from 1999–2001. As officer of the ESP, I helped Jean Alexis Grimaud to create the EuroCellPath (European Courses in Cellular Pathology) and Gianni Bussolati and Mauro Papotti to establish and develop the European School of Pathology. At first we were involved in giving courses at the headquarters in Turin and afterwards we created branches in Poland (Warsaw and Krakow), Turkey (Ankara), Romania (Craiova), Czech Republic (Hradec Králové) and elsewhere, where we continue to teach the next generation of pathologists from around the world – promising students and residents, in particular from resource-poor countries. I believe the mobility of these students for the next 10 to 20 years will help pathology retain its importance

Will pathologist consultations with patients help?
I think it's important for pathologists to communicate with patients; I personally consult with patients or their relatives via email every week and my colleagues and I meet regularly with patient associations. We are crucial to supporting the understanding of a diagnosis. But I think talking with patients is an entirely different skill set to the one we use in our day-to-day work – and it's one that can be very difficult for pathologists. If I have a patient with a terminal condition, for instance, I personally wouldn't know how to communicate that diagnosis to them. I think that's a task best left with the clinicians.

What's your prediction for the future of pathology?
Our future will depend on the education and training of medical students. This is absolutely critical. Pathology is the only discipline in the medical curriculum where you can integrate the clinical aspect with the molecular and genetic aspects of disease, and this is vitally important for understanding basic medicine. Pathology will survive if we can retain pathology departments within hospitals and universities. I predict that there will be fewer pathology departments in the future, but those that do exist will be very large.

I was recently talking with the Portuguese Minister of Health and I told him, "If you want to keep good pathology departments in our hospitals, you have to pay better and provide good career paths. But you should concentrate on joining together the pathology departments of other hospitals, and not dispersing them."

You have achieved a great deal during your career. You have educated thousands around the world, made numerous discoveries, received countless awards, and inspired a generation of pathologists. What is your greatest achievement?
Teaching people to be better than I am. Over the years, I have received so many nice letters from students of ours from around the world (Turkey, Brazil, Algeria, Serbia, Chile, the list goes on…) thanking me for our hospitality. It's always an achievement for me to see people going further than I have – like my colleagues at IPATIMUP, who are now considered world-class experts in gastric cancer.

IPATIMUP, while it's a collective effort, is a big personal achievement for me too. Seeing many of my students excel and become leaders in their respective fields, some of whom have made practice-changing scientific discoveries that impact patient care globally, makes me feel very proud.

Linking with students, society, the media; for me, that's what I love about my career, and it's so important. The fact that I like my job so much, though, means that I am totally terrified at the prospect of being forced to retire from the university in two years' time, when I turn 70!

Though retirement from university work is on the horizon, surely you still have many years ahead in pathology. What are you planning to do?

I want to focus my efforts on the new institute that we have just opened in Porto. That's the most important thing for me now: to further the fields of cancer (which will go on being my main interest); host interaction and response; and neurobiology and neurological disorders, which are the three key focuses of the institute. I will be happy if I can help build this new institute like I have done with IPATIMUP over the last 25 years.

How did it feel to be nominated #1 on The Pathologist Power List?

I was very, very happy. But I must confess – as I stressed previously, almost every person in Portugal has a cell phone, but I don't. I would class myself as computer-illiterate. I always read The Pathologist, but in print, so I didn't even realize the competition was happening. I got an email from Mike Wells, saying "Congratulations, Manuel, you won!" I didn't know what he was talking about. It was a totally unexpected surprise!

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The Many Faces of Follicular Lymphoma

Follicular lymphoma is currently viewed as a single disease entity – but will recent pathologic findings redefine our perspective?

By German Ott

Follicular lymphoma (FL) is the most common type of indolent non-Hodgkin lymphoma in the Western hemisphere, and is regarded as a well-defined disease entity. Its clear morphology, immunophenotype and genetic background make diagnosing the disease relatively simple. But recent developments are changing our perceptions of FL, and could affect the prognosis and management of the condition. Is it time to change our thinking?

At a Glance

- *Follicular lymphoma (FL) is a well-defined disease with straightforward diagnostic criteria, a clear genetic background and defined precursor condition – which means that diagnosis is generally feasible and reproducible*
- *Recent discoveries are challenging the idea of FL as a single disease entity; one workshop gathered cases with a range of unusual features that defy classical diagnostic criteria*
- *Pediatric FL, which exhibits very different features to the adult forms of the disease, adds yet another dimension to the challenge*
- *Pathologists must stay abreast of new discoveries and definitions regarding the heterogeneity of FL so that they can contribute valuable information to treatment decisions*

Diagnosis might seem simple…

In 80 to 90 percent of FL cases, the affected lymph nodes display destruction of the regular parenchyma by atypical follicular structures formed by components of the normal germinal center (GC) – centroblasts and centrocytes. In contrast to the reactive GC, the neoplastic follicles are devoid of regular zonation, with a dark zone mainly consisting of blasts and a light zone largely composed of centrocytes. They also show a predominant infiltrate of centrocytes, with a small or medium number of interspersed centroblasts. Aside from these typical morphological changes, the fundamental biological difference between neoplastic and reactive follicles is the expression of the BCL2 oncoprotein in the neoplastic follicles. This provides an ideal diagnostic tool for proving the neoplastic nature of atypical GCs, and expression of this anti-apoptotic protein is the biological hallmark of FL. BCL2 expression is downregulated in all reactive conditions, permitting GC cells to die if the B cell receptor structures on their surfaces are not optimally fit for antigen recognition and/or antibody formation. In more than 85 percent of FL cases, the overexpression of *BCL2* – not seen under normal physiological conditions – is mediated by formation of the t(14;18)(q32;q21) chromosome translocation. Molecularly speaking, this means that enhancer elements of the B cell receptor heavy chain *(IGH)* gene promoter in 14q32 are juxtaposed to the *BCL2* oncogene in 18q21. Formation of this *IGH/BCL2* chimeric gene leads to constitutive overexpression of the BCL2 protein in the GC, prolonging the lifespan of suboptimal cells and permitting the acquisition of more alterations, eventually leading to lymphoma.

It is also interesting to note that, depending somewhat on age, around 50 to 70 percent of healthy adults have been shown to carry t(14;18)-positive "FL-like" B cells in the peripheral blood, although in very small percentages. These circulating *IGH/BCL2* positive cells are thought to represent the possible soil of lymphoma development, although t(14;18)-carrying patients have only a 5 percent lifetime risk of developing overt lymphoma.

In our work as pathologists, the earliest morphological alteration we recognize related to circulating FL-like B cells is "FL *in situ*," or "*in situ* follicular neoplasia," in which individual GCs are partly colonized by *BCL2*-positive B cells harboring the t(14;18) translocation, but show no signs of truly invasive disease. So at first glance, FL appears to be a lymphoid cancer type that is very uniformly defined by its morphology and immunophenotype, has a unifying genetic background, and evidences a clearly defined precursor condition – making diagnosis both easy and reproducible.

…but is it?

Despite the classical hallmarks of FL, the idea that it is a uniform disease has been severely challenged recently. Clinicians have known for some time that the disease can present with astonishingly diverse clinical courses – some patients survive for decades, while others succumb to the disease within just a few months. At the same time, pathologists have started to recognize a remarkable heterogeneity in FL's morphological features. A workshop organized jointly by the European Association for Haematopathology (EAHP) and the Society of Hematopathology (SH), "Redefining the spectrum of small B-cell lymphomas in light of current technology" (1), has assembled an extensive collection of FL cases (as well

as other indolent B cell lymphomas) characterized by a plethora of unusual features that deviate from the disease's classical definition. The cases, presented by contributors from all over the world, showed all sorts of peculiarities related to morphology, antigen expression patterns, and genetic features of the tumors (2). FLs with a predominantly or entirely diffuse growth pattern, or with features related to monocytoid and marginal zone differentiation of the tumor cells within a follicular background, were presented. Antigen expression patterns varied widely, with the typical GC markers CD10 and BCL6 shown to be present in only a subset of cells – or not at all – requiring the use of other GC markers to arrive at the correct diagnosis. Crucially, many of these cases were found to lack the prototypic t(14;18) chromosome translocation, and these tumors also frequently exhibited particular – and deviating – morphological and/or immunophenotypic features.

"Mounting evidence suggests that no therapy at all may be needed in distinct FL variants."

The riddle of pediatric FL

Along with the curious cases presented at the EAHP-SH workshop, another exception to nearly all of the rules are FLs that develop in children or young adults – the so-called "FL of pediatric type." In contrast to their adult counterparts, these clonal lymphoid tumors usually present with localized disease (clinical stages I or II) and have an excellent prognosis, with most patients successfully treated by simple excision of the affected lymph node(s). The majority of cases are composed of medium-sized and large GC cells (grade 2–3), are *BCL2* expression negative, and are also devoid of the classical *IGH/BCL2* rearrangement.

It is perhaps pediatric FL that most profoundly challenges our ideas about the uniform classification principles of lymphoid diseases. According to the World Health Organization Blue Book rules, lymphoid diseases should have a common morphological pattern, so that different pathologists can use the same methods to recognize them. They should also have a comparable clinical background, so that they can be recognized by clinicians. But we are becoming increasingly aware that in many, if not all, lymphoid cancers (not to mention other cancer types), there is a growing awareness of heterogeneity that is beginning to dramatically affect our treatment approaches. Although FL presenting with localized disease may be treated – successfully, in a sizeable proportion of cases – by radiotherapy without systemic chemotherapy, mounting evidence suggests that no therapy at all may be needed in distinct FL variants, including FL of pediatric type.

Time for a change?

The pivotal question for pathologists is whether it is justified to regard – as we currently do – a disease with such a tremendous spectrum of morphologies, biological features and clinical presentations, as just one disease. For the time being, the current practice of defining FL as one disease with differing subtypes and variants, possibly characterized by a distinct clinical course, seems to be justified, but this attitude may change. Newer insights into the diversity of lymphoid proliferations have already influenced taxonomic principles. In the forthcoming update of the 4th edition of the WHO classification of tumors of hematopoietic and lymphoid tissues, it has been proposed that the term "FL *in situ*" indicating a malignant tumor, should be changed to "*in situ* follicular neoplasia," taking into account the low progressive potential of the lesion. If adopted, this new definition will add a new dimension to the question, "to treat, or not to treat?" and ensure that clinicians are even more aware of the indolent nature of the condition.

It is of pivotal importance for pathologists to stay familiar with the expanding spectrum of follicular lymphomas. Treatment for FL is likely to involve very meticulous decision-making, depending on the recognition of crucial traits that characterize lymphoproliferations with tremendously different grades – from the very indolent to the very aggressive. At the same time, it will be our task to unravel the molecular basis of these differences in order to better classify these often enigmatic lymphoid proliferations.

German Ott is professor of Pathology and head of the Department of Clinical Pathology at the Robert-Bosch-Krankenhaus in Stuttgart, Germany.

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A Cure-All For Block Management Woes?

Automated paraffin block storage systems can increase traceability and security while reducing the time and effort needed to sort and store

By Valérie Costes Martineau, Laure Dumas and Nicolas Leventoux

In an era of increasing digitization, it's rare to have a conversation about physical storage. But it's an area that needs our attention – the volume of paraffin blocks needing storage has increased dramatically over the last 10 years and is continuing to rise. The population is growing, patients are aging, cancer incidence is rising, and standards of care are improving – all things that lead to an increase in tissue sampling. But with tighter legal frameworks, more exacting standards for laboratory accreditation, and the increase in blocks circulating for second opinions or advanced analysis, there's a desperate need to manage paraffin block storage and access. Especially given the current shortages of technicians and resources, there's a real risk of mislabeling or misplacing blocks – and, because they're the ones that most often change hands, it seems that it's always the most precious blocks that go missing.

Each error or loss of a paraffin block risks delaying key diagnoses and treatments. This pressing need, and the lack of an established management system, led to a collaboration between the medical device department at CHRU Montpellier and Dreampath Diagnostics to develop FINA, the first fully automated paraffin block management system. Our two groups cooperated to determine the necessary specifications and to test each prototype. The final version of FINA, which we've been running routinely in our lab since June of 2014, consists of a block scanner and accompanying management software.

At a Glance

- *The volume of paraffin blocks in use has seen a dramatic increase – but our storage space hasn't*
- *Complicated, non-standardized block storage systems can lead to errors and losses, which in turn can delay vital patient care*
- *A fully automated paraffin block storage system reduces both the time and space needed to sort and file large numbers of blocks*
- *The system can also improve security by limiting access to storage areas and tracing each individual block's whereabouts*

Manual management

CHRU Montpellier is a university laboratory that handles about 30,000 biopsies and surgical samples each year and produces about 100,000 blocks per year. In the last 12 months, we have retrieved 19,712 blocks from storage – mainly for daily routine techniques (91 percent), but also for targeted therapy (4 percent), research or clinical trials (3 percent), and access to our expert network or second opinions (2 percent). Before the advent of FINA, these blocks were sorted by numerical order in metal drawers, a system that required a lot of time and space. Anytime a large series – like an autopsy, fetal pathology, or set of bone specimens – came in late, we had to move a large number of blocks. Once a week, we transferred blocks from the drawers into a separate set of cardboard drawers for long-term storage in the basement. The system was difficult to use; there was no security, we only used a block register when we knew samples would be out of the lab for a long time, and our technicians lost a lot of time looking for blocks that had been misplaced or removed and then re-archiving them after use. Just managing our blocks required the equivalent of nearly one and a half full-time jobs!

Automated management

To gain more control over our blocks, we turned to automated management. The FINA system consists of a scanner, a computer with a PDA, a label printer, and a set of cabinets (Figure 1a). With this system, the blocks are stored in a special tray at the cutting station; it holds 240 blocks, but they can be placed in random order to save time (Figure 1b). Once the technicians have finished cutting, they scan the tray (Figure 1c) to enter it into the specialized software, which requires a user password to ensure access security and generates reports to improve traceability. Scanning time varies from two to six minutes based on the number of blocks, quality of printing and cleanliness of the paraffin block. The percentage of unread blocks is very low (in our case, 242 of 26,400, or 0.9 percent), and chiefly occurs as a result of paraffin deposition on the barcode of the label. If a barcode is damaged or unreadable, we also have the option of taking a photograph and entering it manually into the software.

To "check out" a block, the user creates a pick list and exports it to the PDA (Figure 1d). For each block, the system asks the user's name, reason for removing the block, and expected duration of use. The PDA shares this information with the main system computer; then, if the blocks aren't returned on time, alerts and reports help us to follow up with users and ensure the samples don't go missing.

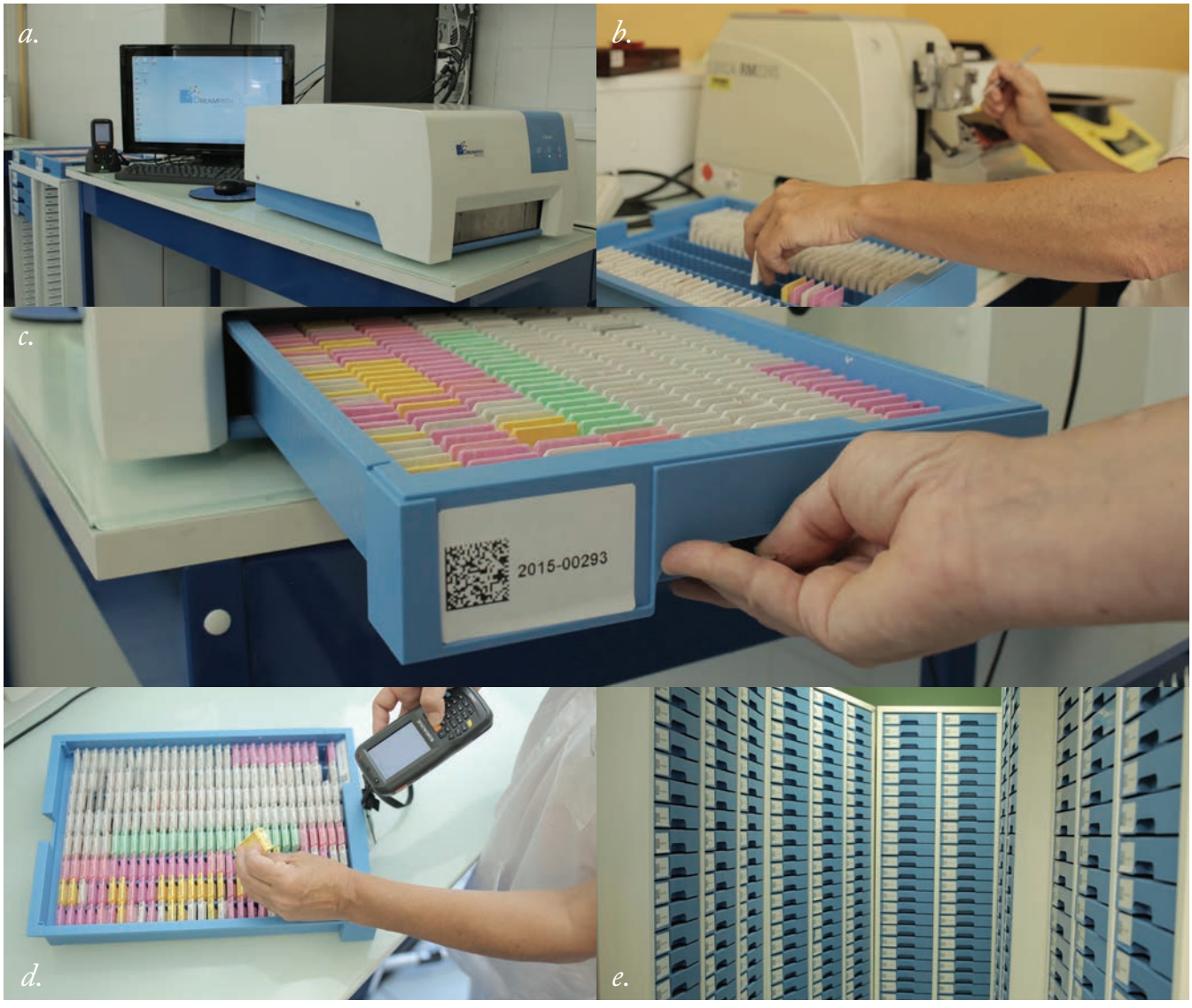

Figure 1. a. The FINA paraffin block storage system. b. Blocks randomly placed in a storage tray. c. A complete storage tray entering the scanner. d. Using the PDA to remove a block from storage. e. Our laboratory's block storage cabinets.

We also have FINA connected to our laboratory information system (LIS), so that we can automate our requests directly from the LIS. We store each tray in a cabinet in the laboratory for two years (Figure 1e) before transferring it to long-term storage – at which point, the FINA software records its new position.

Using a fully automated system not only saves us the space needed to sort blocks, it also allows us to ensure good traceability by recording who checks out each block, when, and why. It minimizes the risk of loss, as we can now better manage the blocks as they move within and outside the lab. The use of passwords and reports improve security, as only authorized people have access to the blocks and the system records all activity. But although all of these are useful functions, the one we've found most useful in our lab is that one entire person's worth of workload has been taken off our hands, letting us devote our most valuable resource – time – to our work.

Valérie Costes Martineau is head of the department of anatomic and cytologic pathology at CHRU Montpellier, France.

Laure Dumas and Nicolas Leventoux are technicians in the department.

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Printed Pathology

A 3D-printed prototype in vivo microscope may make an expensive technology cheap and customizable

By Hany Osman

When talking about microscopy, we often hear about technological advances that enable better imaging. Higher resolution, increased clarity, and better image processing are all good news – but some advances, like *in vivo* microscopy, seek to change not the outcomes of the imaging, but the tools and techniques themselves. There are clear benefits to *in vivo* pathology – for instance, the ability to directly visualize histology without fixation or processing, the potential to aid in gross examination and frozen sections, and the ability to examine tissues that can't easily be biopsied or cut for frozen section, such as bone or fat. So why aren't more pathologists using it?

At a Glance

- *In vivo microscopy may help with gross examination, frozen sections, and tissues that are difficult to biopsy*
- *It's challenging for pathologists to access the technology though, largely because of its high cost and also the lack of desire of manufacturers to market their wares to lab professionals*
- *I devised a prototype 3D-printable fluorescence in situ microscope that can be used to observe living cells and "uncuttable" tissues*
- *Accessible in vivo microscopy offers pathologists an opportunity to advance our profession and improve patient care*

At the moment, there's a wide gap between commercially available *in vivo* microscopes that are designed for clinical applications and the microscopes pathologists actually use. The current *in vivo* options are prohibitively expensive – and more than that, when I approached a large supplier of endoscopic microscopes, they seemed uninterested in marketing the technology to pathologists as clinicians already had direct access to patients. But these devices give us access to new information, letting us examine the microscopic features of lesions in a minimally invasive way. I believe that's why the *in vivo* pathology and endoscopy market is only growing slowly – and I think that this technology should be in the hands of people who are trained to interpret microscopic features and can therefore access its full potential.

A pioneering prototype

Given the high cost of *in vivo* microscopes and the fact that pathologists aren't seen as a target market, how can we gain access to these tools? Upon closer examination, I realized that the basic idea of the fluorescence *in situ* microscope was simple enough that I might be able to build one myself. I just needed to figure out how to attach an imaging fiber optic to an epifluorescent microscope, the design of which is readily available online. After some research, I came up with a much simpler design. But as soon as I started putting it together, I ran into a problem: even the individual parts of the microscope were too expensive for a resident like me. Rather than give up, I found inspiration in the form of 3D printing. With a little creativity, most of the structural components of the microscope could be 3D-printed. In fact, that proved to be an even better option than sourcing prefabricated parts, because its ability to accommodate different sizes and shapes gave me the freedom to use any optical parts I could buy or salvage (Figure 1).

To build my prototype, I bought the cheapest 3D printer available – a startup cost of US$500. Then I started the design and printing process, which was the most difficult and tedious part of the work. It took almost a full year and hundreds of printed attempts before I settled on the final version of the microscope.

The design is simple – it's a fluorescent microscope with an attached fiber optic probe. The light source is an LED, which I bought from an aquarium supplier, that emits light within a certain wavelength range. It's contained within a 3D-printed housing that holds a collimator, filter, and heat sink for cooling. The emitted light travels through the filter and a lens, reflects off a dichroic mirror into a 20X objective, and is then carried through the imaging fiber (a bundle made up of thousands of microscopic fibers, each of which acts as a pixel that transmits light, contained in an adapter I designed) to the tissue (Figure 2).

Because this was a fluorescence microscope, the tissue still needed to be prepared for imaging. I applied acridine orange, a dye that non-specifically stains the nuclei of living cells by binding to DNA. When illuminated with cyan light (502 nm), the labeled nuclei emitted green light (525 nm) that was carried back through the microscope to a consumer digital SLR camera I attached. Those images could then be presented on a screen or laptop. The image resolution from the prototype microscope was good enough to allow for accurate 3D architectural evaluation of tissues (Figure 3).

Homegrown versus high-tech

Of course, there are distinct differences between this prototype and commercially available *in vivo* microscopes. One

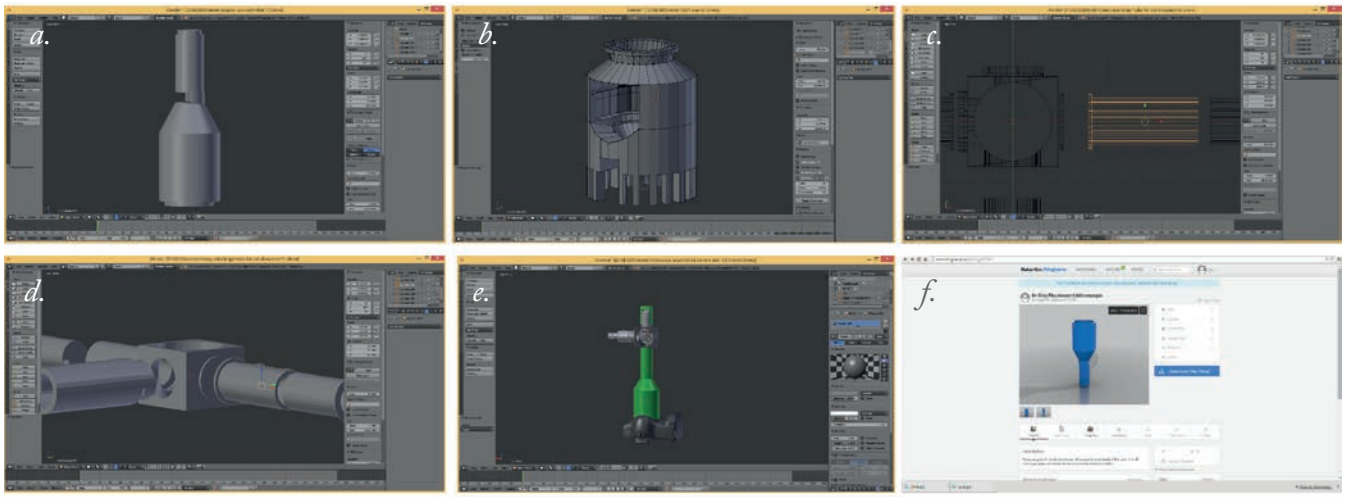


Figure 1. Microscope designs created in Blender, a 3D graphics program. a–e. Renders of a. the camera tube, b. the light source, c. the filter cube, d. a partially assembled microscope, and e. the fully assembled microscope. f. The completed and freely available design for the imaging fiber adapter.

is that the commercial model is a confocal microscope that images tissue in 2D slices. My microscope lacks that capability – which I think is an advantage, albeit an unintentional one. The cellular resolution in both technologies is too low to allow for accurate diagnosis, so the inability to optically section the tissue leads to 3D (native state) rendering of the tissue. As a perspective pathologists don't usually see, this adds valuable information. There's another difference; commercial microscopes use intravenous contrast that illuminates the tissue indirectly and also demonstrates vascular leakage if present – unlike my microscope, which uses a contrast agent directly applied to the tissues. It's a technique that can be applied to cells *in vivo* using non-damaging agents like fluorescein.

I've found that I can observe a lot through *in situ* evaluations of pathology specimens when compared to histologic sections. Some cytology preparation methods, such as Papanicolaou smears, provide superior cellular and nuclear cytologic detail, but lack in architectural aspects when compared with classic hematoxylin and eosin (H&E) sections;

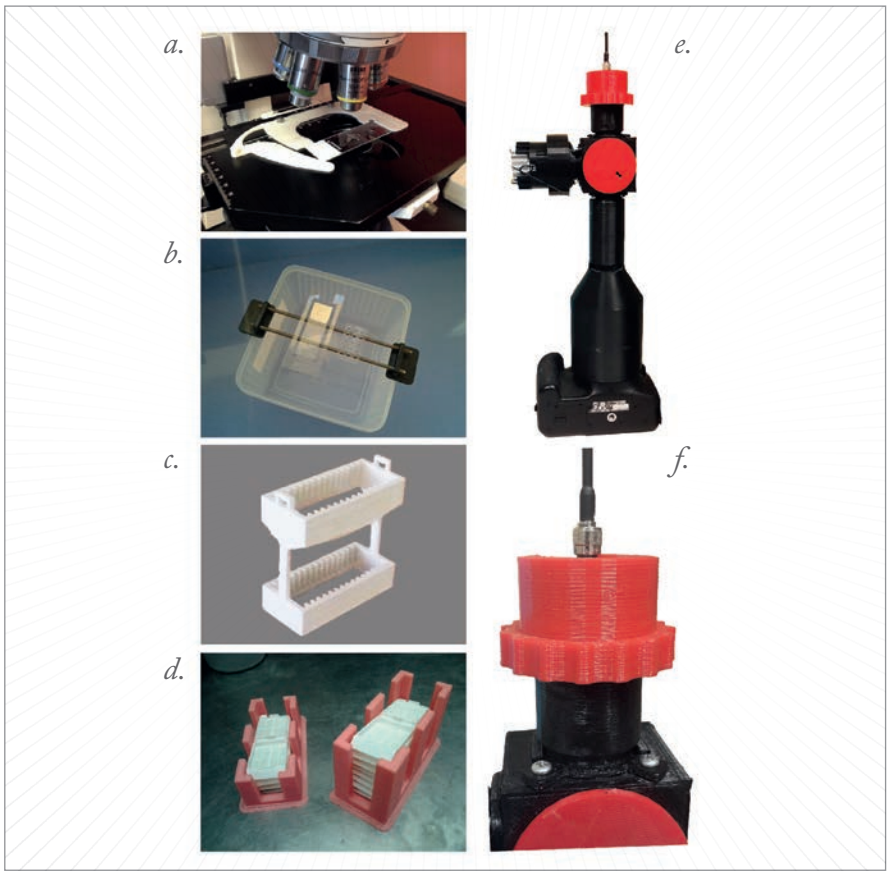


Figure 2. a–d. Completed 3D-printed tools for microscopy, a. stage slide holder, b. slide rack, c. slide holder for staining, d. block holders. e. The complete 3D-printed microscope. f. A close-up view of printed parts like the fiber holder.

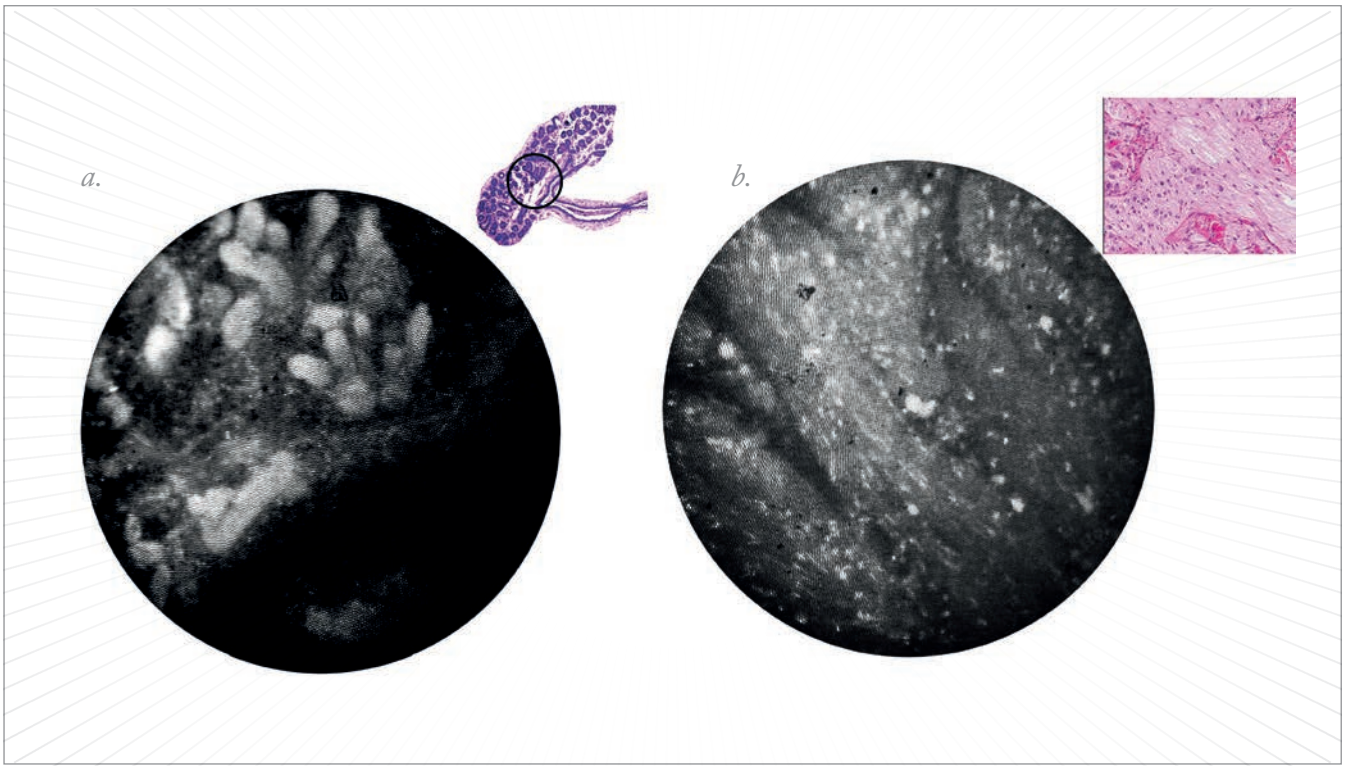


Figure 3. Tissue sections of a. benign breast lobules (see inset) and b. ganglioneuroma (see inset) observed using the 3D-printed microscope.

similarly, *in vivo* microscopy can provide architectural detail superior to H&E sections, but yields less cytologic information. Imaging normal breast tissue (Figure 3a) yielded a good example of this; using my microscope, I was able to see that the breast lobules were actually oval- and sausage-shaped in three dimensions – a fact I wasn't able to appreciate by looking at conventional 2D sections.

Facing the future

We're currently investigating several potential applications for the microscope. Its main potential lies in guiding sampling for gross examination and in frozen sections – offering a possible new solution to screening for dysplasias or diagnosing conditions like ulcerative colitis. It can also be used to evaluate margins, determine the dimensions of tumors, and confirm the involvement of structures like lumens or lymph nodes at grossing. Pathologists can observe "uncuttable" tissues like bone or fat, or even conduct pre- and intraoperative histologic evaluations. The use of specific fluorescent antibodies may enhance the diagnostic capabilities of the microscope, allowing easier determination of tumor margins. The microscope is not without its limitations, of course; there's a defined screening surface area, not all tissues produce good fluorescence, and the images can be complex and the learning curve steep. But in cases where these obstacles can be overcome, grassroots fluorescence *in situ* microscopy has the potential to become a cheap and efficient alternative to conventional pathology.

I believe that, if more pathologists get proactively involved, we'll see more of these kinds of clinical transitions in pathology. Radiology is a great example of this – technological advances made radiologic imaging and interpretation accessible to physicians of all specialties, so radiologists secured their value by taking initiative and developing outstanding interventional procedures. I think that, as *in vivo* microscopy advances and spreads among clinicians, pathologists will face a similar challenge – and I think this is a great opportunity for pathologists to become familiar with and advance *in vivo* pathology to a point where it becomes relevant in patient care.

Hany Osman is a fourth-year resident in the Department of Pathology and Laboratory Medicine at Indiana University, USA.

Benchmarking Companion Diagnostics

What does analysis of the last five years of literature on companion diagnostics tell us about the priorities of the field, and the contributors to it?

By Michael Schubert

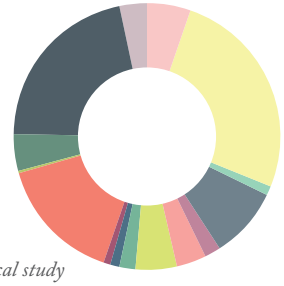
New medical interventions often get a lot of media attention – but less is given to companion diagnostics, diagnostic tests and devices that provide essential information for the use of a corresponding intervention. But companion diagnostics are vital to the safety and efficacy of those interventions; they help physicians decide which patients should receive a particular therapy, which patients need to be monitored more closely, and which patients are responding appropriately to treatment.

But what do those "on the ground," developing and using companion diagnostics, think? What are your peers reading and researching? To find out, we decided to benchmark all of the PubMed-listed literature on the topic. We asked:

- What are the major topics in the field?
- Which journals have the greatest impact?
- How is the knowledge available online?
- What types of articles are being published?
- Who are the most prolific authors?

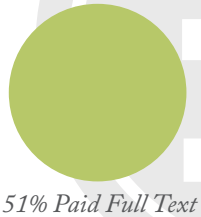
PubMed was searched for "companion diagnostic." The data were analyzed in Microsoft Excel 2013.

Article Categorization by Number of Publications

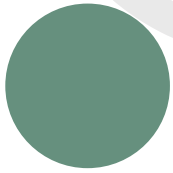


- *352 Clinical study*
- *206 Clinical trial*
- *294 Review*
- *119 Comparative study*
- *74 Basic, animal or preclinical study*
- *69 Cross-sectional study*
- *59 Case report*
- *50 Cohort study*
- *42 Opinion*
- *27 Multicenter study*
- *24 Epidemiological study*
- *15 Validation study*
- *15 Observational study*
- *12 Meta-analysis*
- *5 Practice guidelines*

Fee or Free?



51% Paid Full Text



43% Free Full Text

5% Abstract only

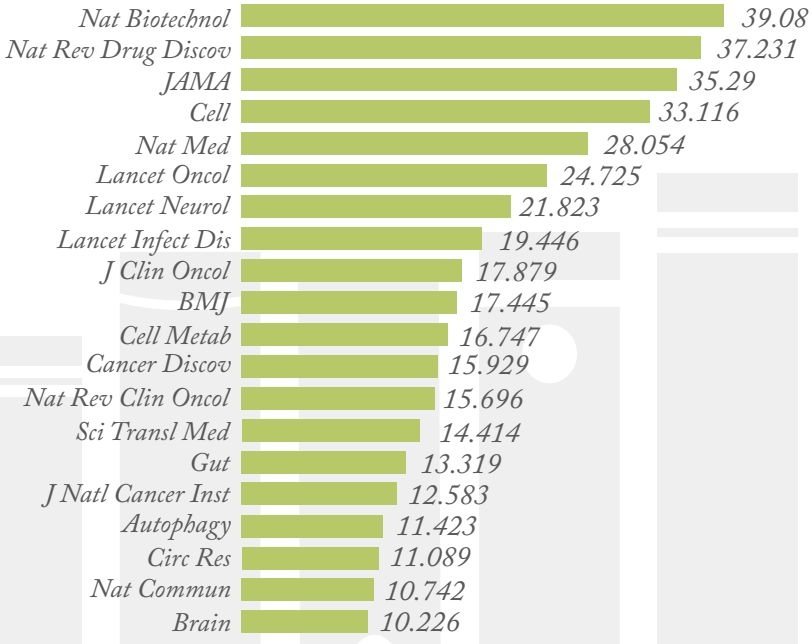
1% Not available online

Top 20 Important Words

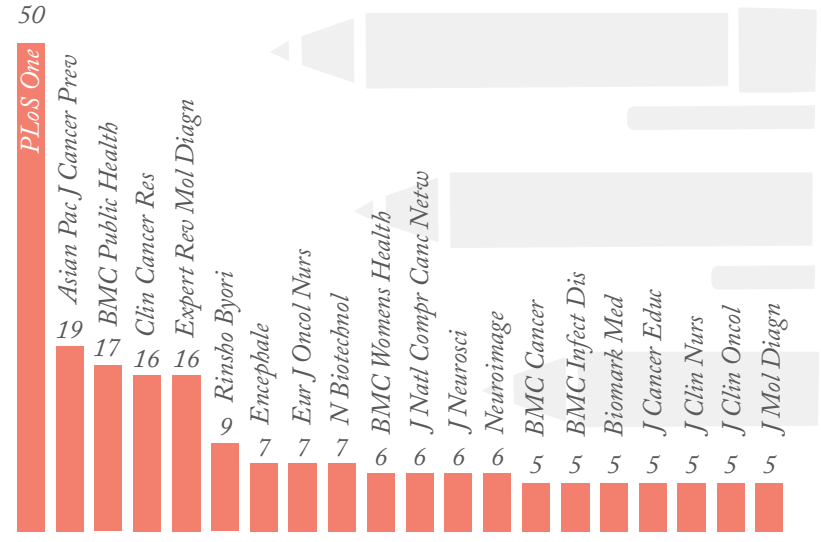
1. *personalized*
2. *BRAF*
3. *lapatinib*
4. *biomarker*
5. *ROS1*
6. *FFPE*
7. *omic*
8. *HER2*
9. *ALK*
10. *targeted*
11. *blaCTX*
12. *VEGF A*
13. *pharmacogenomic*
14. *cetuximab*
15. *PIK3CA*
16. *cancer*
17. *mass*
18. *information*
19. *outcome*
20. *NNDSS*

Here are the top 20 important words found in the literature. Important words have more frequent occurrences in the result subset than in MEDLINE as a whole; therefore they distinguish the result subset from the rest of MEDLINE.

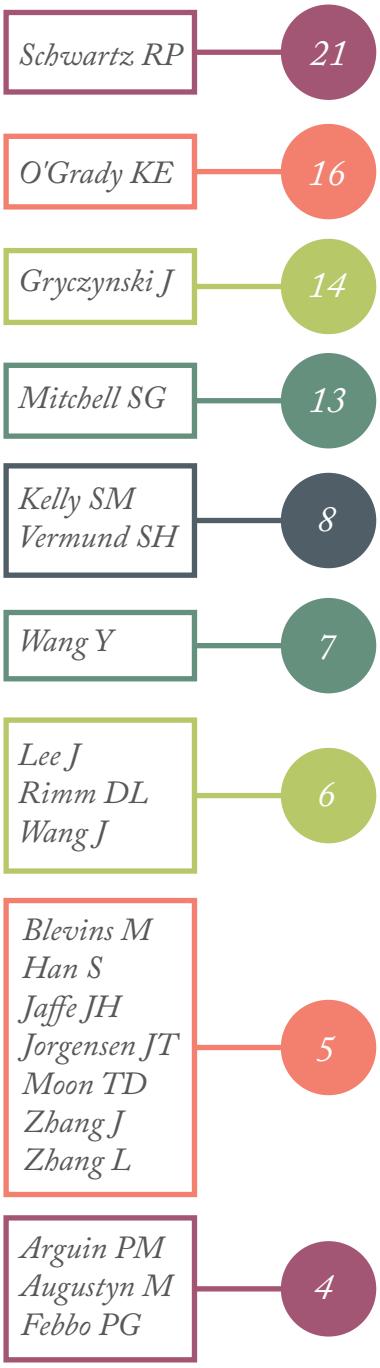
Top 20 Journals by Impact Factor



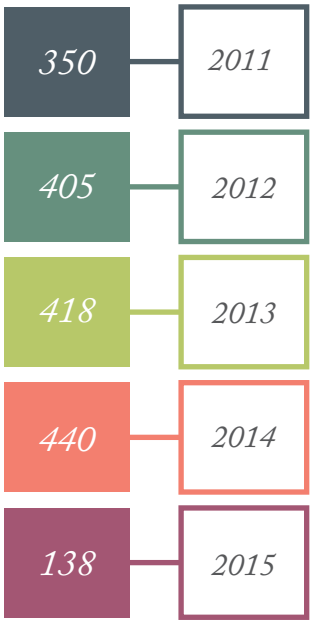
Top 20 Journals by Number of Publications



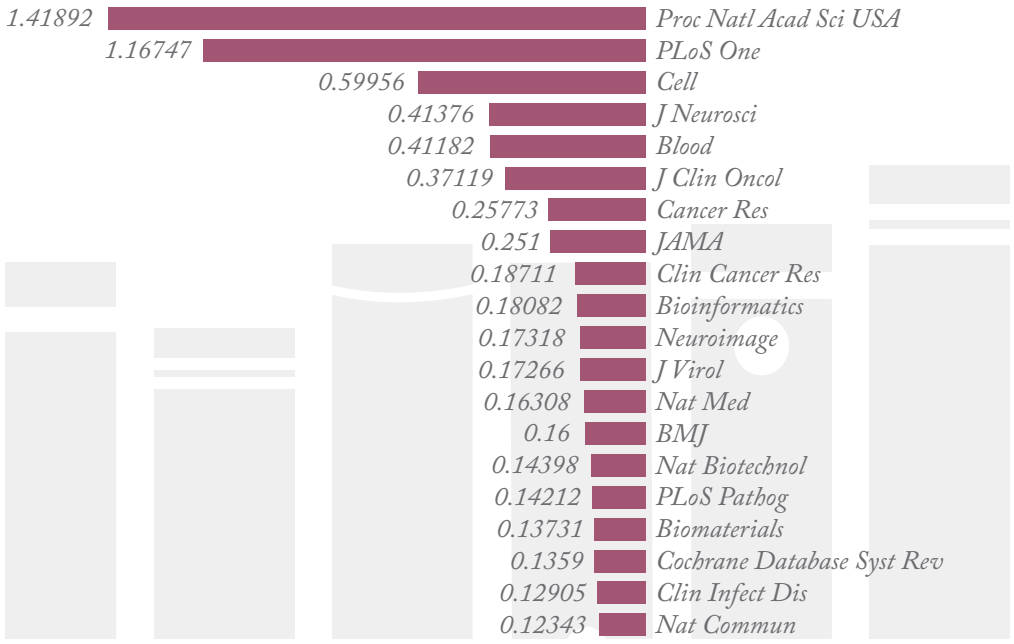
20 Most Prolific Authors



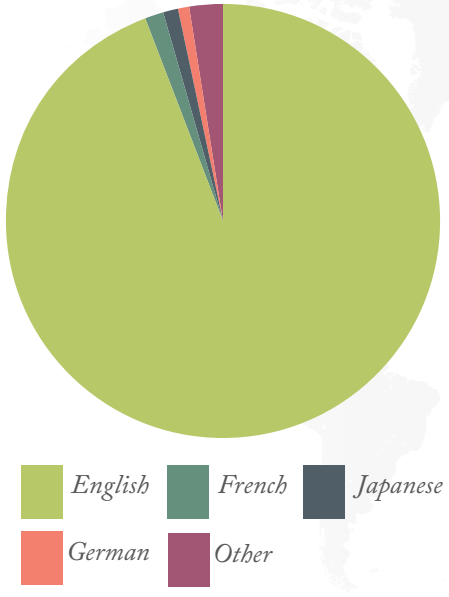
Publications Per Year



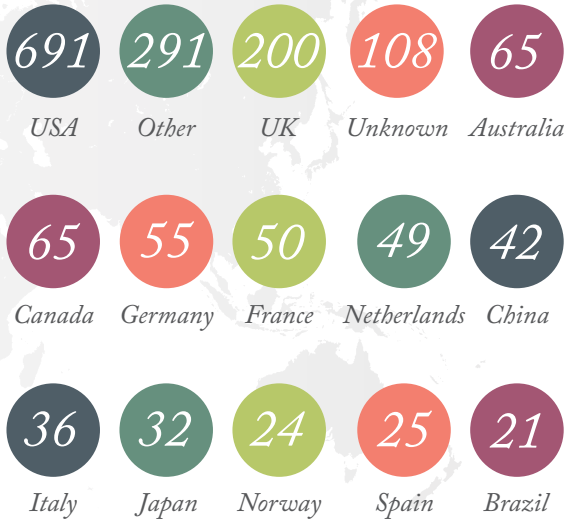
Top 20 Journals by Eigenfactor



Publications by Language



Countries by Number of Publications



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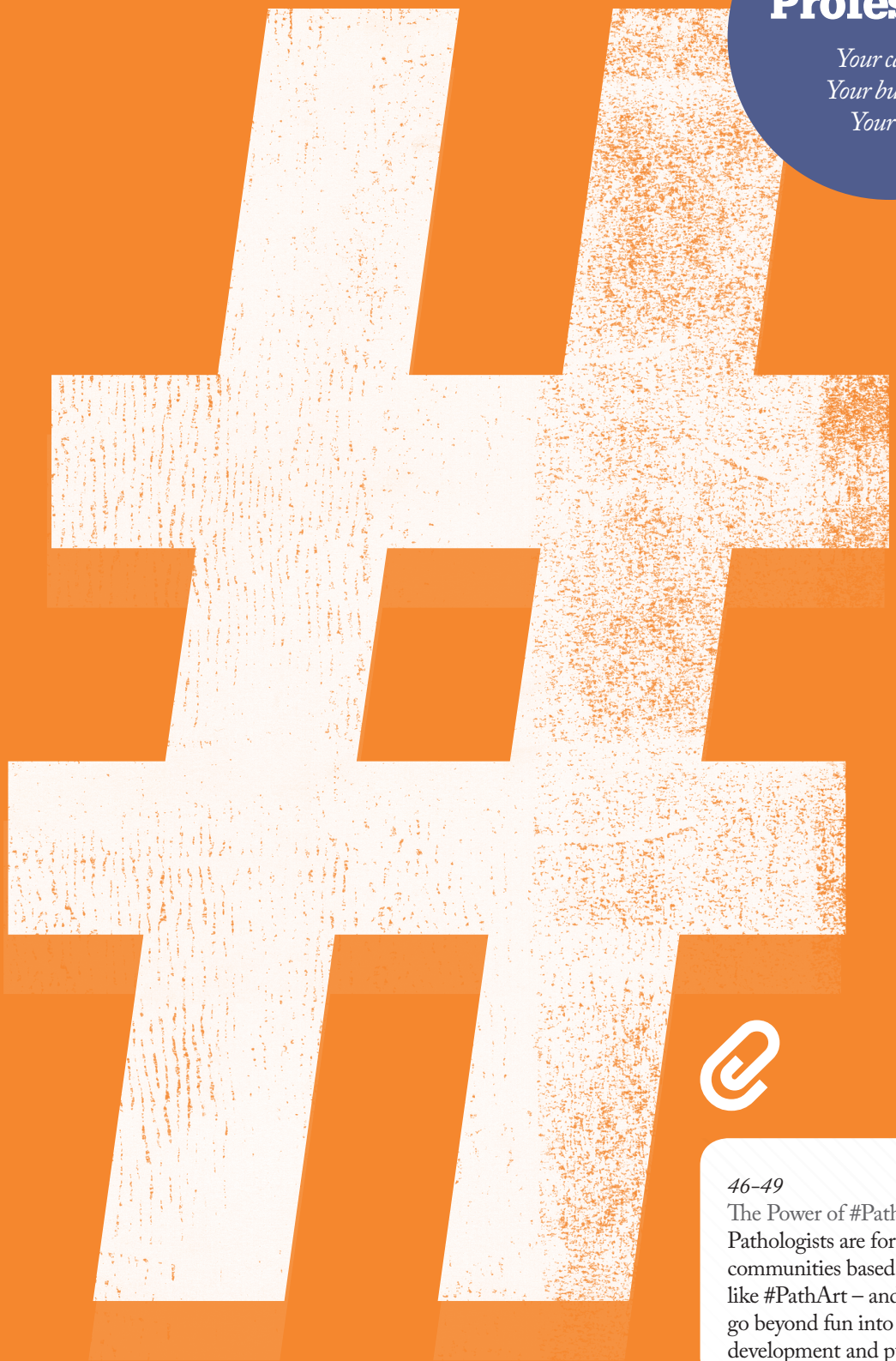
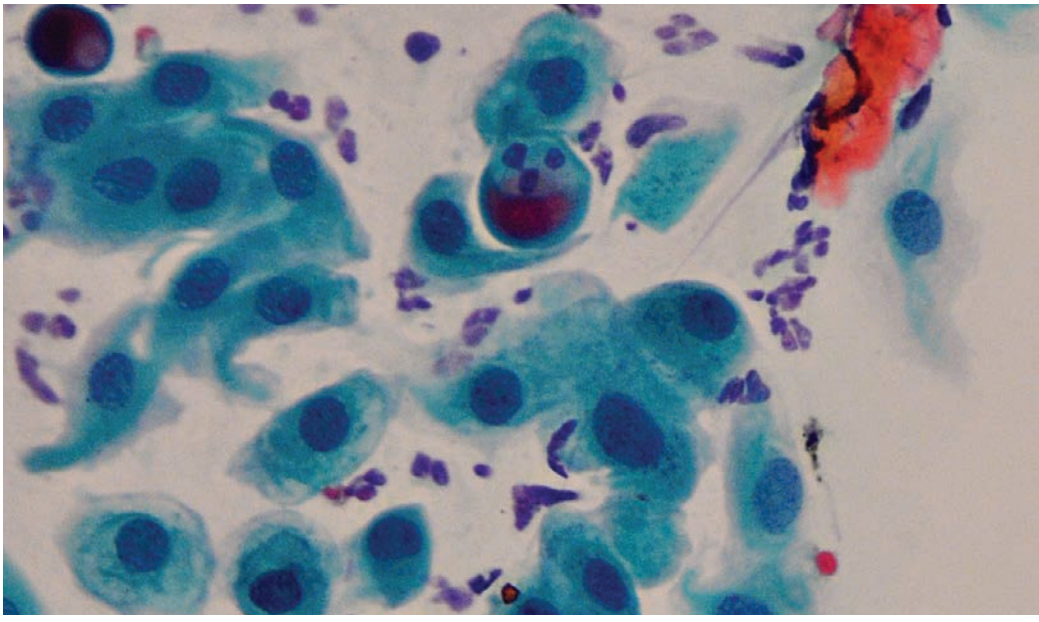


The Power of #PathArt

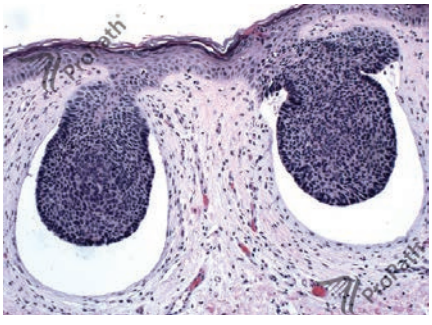
Social media initiatives like the popular #PathArt hashtag draw attention to pathology and help distant lab medicine professionals connect

By Roisin McGuigan and Michael Schubert

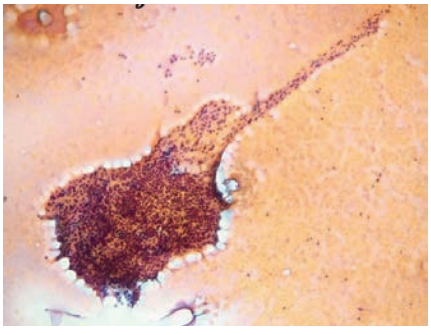
One of pathology's most enduring stereotypes – and one that lab medicine professionals are trying their hardest to overcome – is that of the pathologist as "basement dweller" or "microscope lover" whose main source of joy is an interesting slice of tissue or smear of blood. But pathologists themselves know that this isn't the case at all. In fact, the lab is often a prime example of "work hard, play hard." The work done by lab medicine professionals is as difficult as it is vital, so those who do it enjoy being able to see the lighter side of their chosen discipline while exercising their creativity. And community-building isn't the only benefit of "fun" pathology – it also helps to draw attention to the field and make it more approachable to trainees and the general public.



Parabasal cell with a smile by @NejibY



A basal cell carcinoma looking back, by @RyanHickMD



Thyroid "rock band" cytopathology by @GeronimoJrLapac

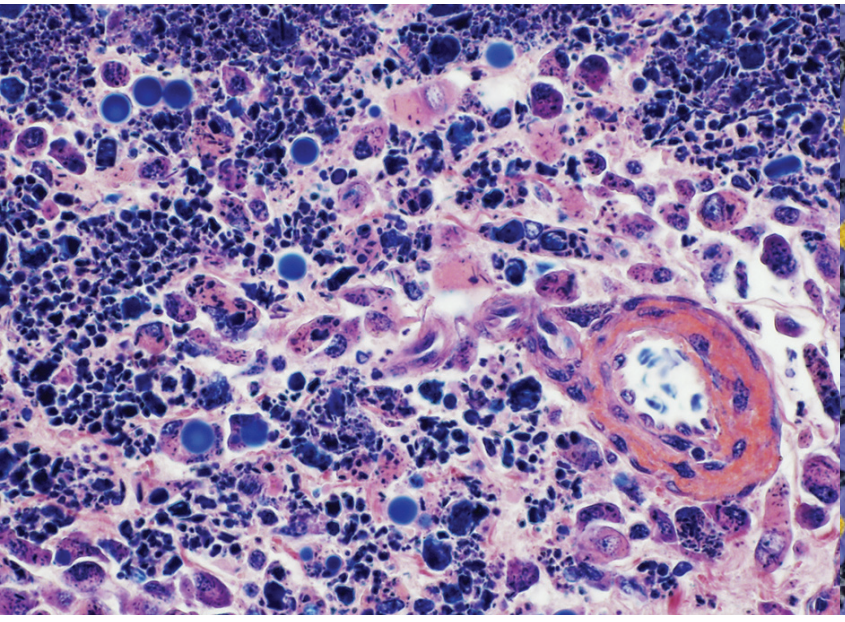
At a Glance

- *Lab medicine professionals have a unique sense of humor – including on social media, with trends like the #PathArt hashtag*
- *#PathArt participants look for recognizable images, usually in tissue samples, and then share them with friends and colleagues*
- *Community-building initiatives like social media hashtags allow pathologists to get acquainted with distant colleagues*
- *Fun, accessible online interactions also draw the public eye – and play a key role in promoting pathology*

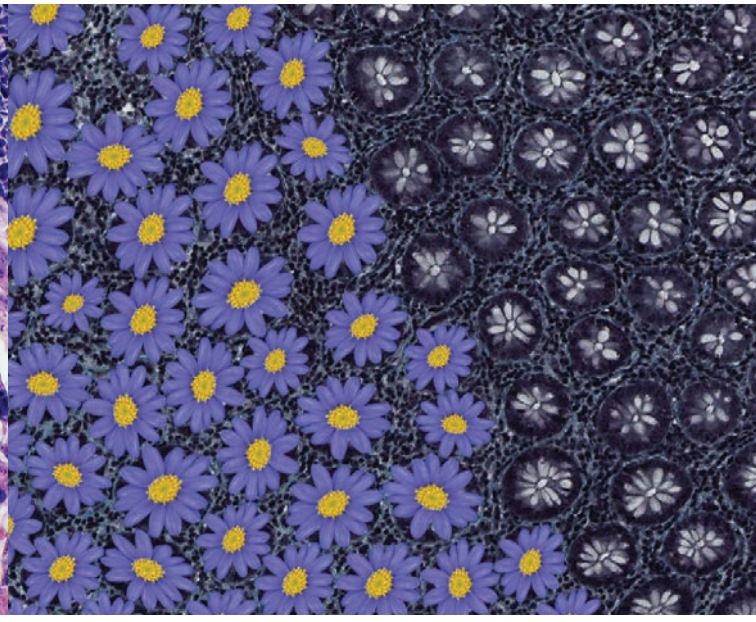
#PathArt with a purpose

A prime example of this lightheartedness is the popular Twitter hashtag #PathArt, created in March 2015 by pathologist and social media guru Jerad Gardner. Countless pathologists use Twitter for personal and professional development. They follow up-to-the-moment news, liaise with professional societies, represent their hospitals and research groups, and find others online who share their interests. And when a group of like-minded pathologists get together on the Internet, what results is a burst of histology-based creativity.

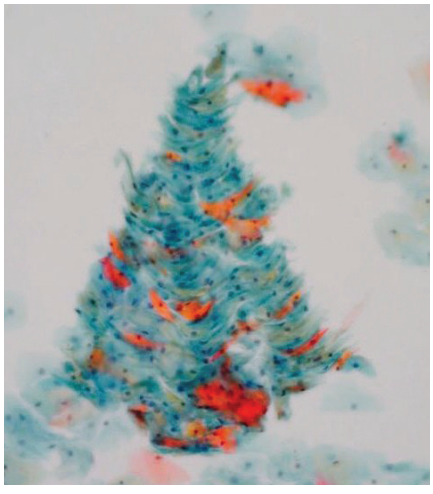
#PathArt images arise when a sample, most often a tissue section, looks like something else under the microscope – anything from a classical painting to the Death Star. What would formerly have been the source of a chuckle for one or two microscope users has now become a popular trend, and the likenesses are getting more imaginative every day.



Massive crystal storing histiocytosis by @evenmariecrane



Colon daisies: this is one posy you may not want to sniff! By @IHeartHisto



Nothing says 'Happy Holidays' better than a Pap smear Christmas Tree. By @IHeartHisto

A tympanic membrane-bow from an otitis media crust by @IHeartHisto

An impressive glandular alphabet, collected by Ivy Clemente and posted by @NormanZerbe

#PathArt is certainly generating a community. Not only has the tag encouraged pathologists to share their images, but it's stimulating discussion as well. Those who use it compliment one another on particularly beautiful images, offer advice on unusual cases, and even debate which popular characters a given tissue section most closely resembles. Far from hiding behind their microscopes, these pathologists are using them as a tool for communication. And not just among professionals – many of the tweets are pitched at a level accessible to outsiders, so that even students and members of the public can appreciate them. pH7, an undergraduate student science blog from the University of Sheffield, UK, recently featured the #PathArt hashtag, along with a selection of their favorite examples (1), showing that even those with no histology training can get in on the fun.

Creating a community

Why is online community-building so important for pathologists? Medicine is becoming ever more globalized. Where you might once have been limited to colleagues at your institution for a consultation, or had to lose days or weeks mailing slides to distant locations, you can now send digital images in seconds. Knowing who your worldwide colleagues are, where they work, and what specialties and skillsets they have can be the key to solving a difficult diagnostic puzzle – so the more pathologists get to know one another, even through "fun" interactions,

Satirical Stressbuster

GomerBlog is a medical satire blog whose posts reach medical students and professionals around the world. With headlines like "Surgeon Sends Lunch For Frozen Section" and "ED Consults Pathology on Acute Abdomen Just to 'Make Them Aware'," their articles are read, shared and commented upon by pathologists at all levels – usually because they strike a chord. We spoke to Doktor Schnabel von Rom, one of the blog's founders, to ask how their creative outlet came about.

What prompted you to start GomerBlog?
The idea was created in our anesthesia break room. While taking a break, we started talking about funny satire websites and asked ourselves, "What's out there for medicine?" A quick search didn't come up with many recent or overwhelmingly funny sites, and thus the idea was born. The name "GomerBlog" came naturally, as we're huge fans of the novel *House of God* and wanted a slang term that only healthcare professionals would "get." Once we had the site name, the articles flowed from there.

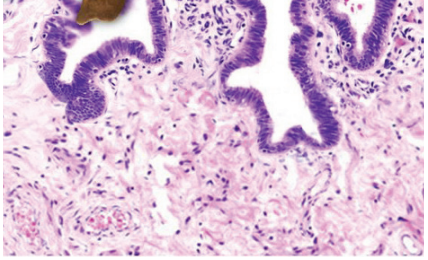
Who are your readers?
We're all over the place – with doctors, nurses, residents, interns, medical students, nursing students, physician assistants, nurse practitioners, and many other healthcare professionals.

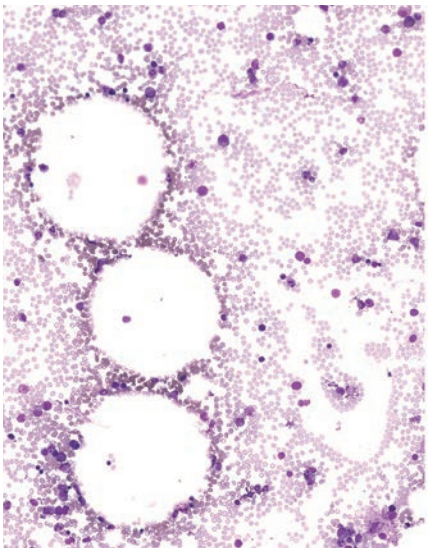
Who are your writers?
Most of our contributors are physicians, but we are always on the lookout for other professions. Of the medical specialties, most of our writers are internists or hospitalists, but we are really all over the place. We have one pathologist, P.E. Coma, who gives us fantastic pathology articles!

Why do you think the blog is so popular?
Healthcare professionals need an outlet. Medicine is stressful and humor has always been a relief valve for many. We hope to provide a smile for a nurse or a chuckle for a physician to make their days just a little bit better. We think that, as medical professionals write the articles, our fans appreciate the realness of the articles because we're right there in the thick of it with them, so they know we really "get it." Most of my articles have come from issues I've actually seen at work.

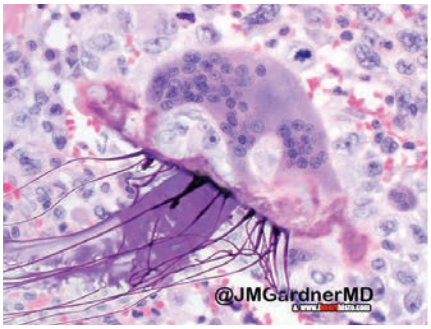
What has the reception been like among pathologists?
I think it has been great. P.E. Coma probably played a significant role in garnering more pathology fans. We received a lot of comments on one of our satirical pieces (http://bit.ly/1nkPl33) about how believable it was. But as with all of our specialties, we like to poke fun at pathology, too. As an anesthesiologist, the paperwork involved with blood product administration is always a touchy subject in our field, so we had to write about it (http://bit.ly/1Q7IOE5 and http://bit.ly/1JMjjXr).

What's your favorite example of pathologist creativity online?
We were totally impressed with the "Thrift Lab" video that parodies Macklemore's "Thrift Shop." (Watch the video at http://bit.ly/1Nq6QaY.) That was one of the greatest satire videos ever.



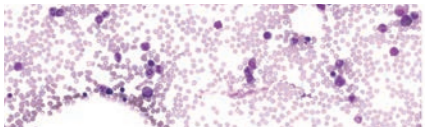


Marrow Christmas & Happy New Smear! A very seasonal red bone marrow smear by @IHeartHisto

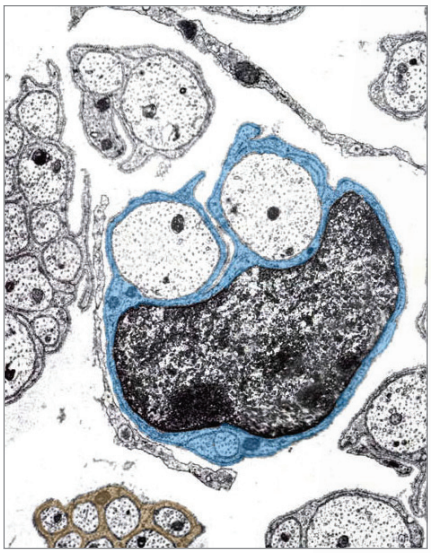


Giant osteoclastic #jellyfish in a sea of malignant osteoblasts by @JeradGardnerMD

Oh deer! It's an appendix testis! By @IHeartHisto



That's no moon, it's a cluster of endometrial cells! By @AmyHDeekenMD



Schwaan cells in non-myelinated nerves look like Cookie Monster in cross-section, by @IHeartHisto

the better. Not only that, but it allows lab-based professionals to reach out to patients and the public.

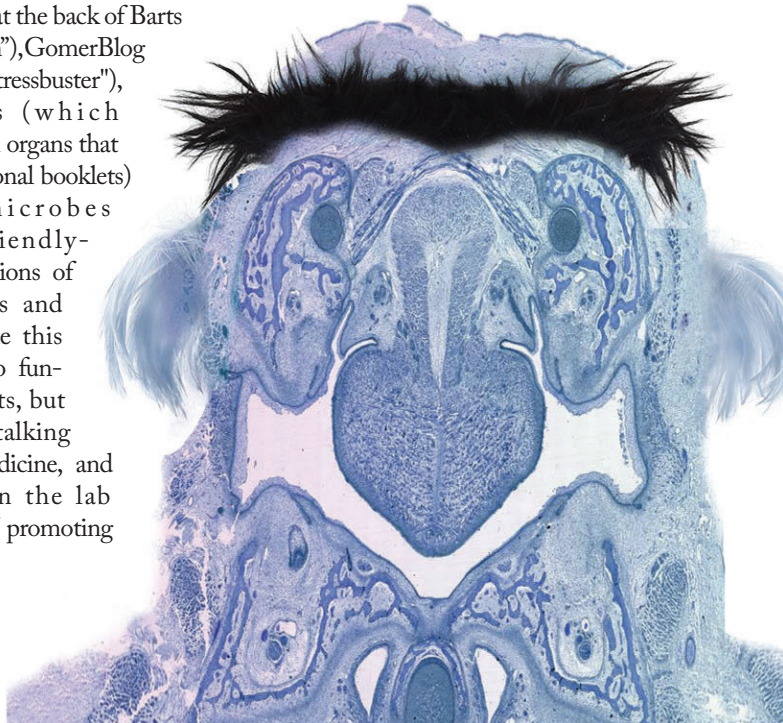
It's as vital as ever to destroy the antisocial stereotypes that plague the field. We've covered the problems these stereotypes cause before (2) – medical students are dissuaded from considering pathology by its reputation, leading to widespread personnel shortages, and patients fail to understand the pathologist's role in their care. It's a problem we hear about again and again; in this issue's interview with Fred Bosman, professor emeritus at the University Medical Center of Lausanne, Switzerland, he told us, "It's important to get the public eye on pathology… We should be much more actively engaged in interaction with the public, teaching them who we are and showing them the importance of what we do." At a time when pathology must move to the forefront of patient care to keep its edge, practitioners can't afford to be held back by misperceptions. Engaging people with interesting facts and visuals in a casual environment like a social media platform is one way of reaching out and showing them what pathology is really like.

Of course, Twitter isn't the only platform pathologists can use for outreach, and #PathArt isn't the only hashtag to explore. Laboratory medicine is slowly making its way into the public eye through initiatives as diverse as the @BellinghamSkull Twitter account (featuring short messages from a skull "hanging out at the back of Barts Pathology Museum"), GomerBlog (see "Satirical Stressbuster"), I Heart Guts (which manufactures plush organs that come with educational booklets) and GIANTmicrobes (showcasing friendly-looking plush versions of human pathogens and more). Things like this not only appeal to fun-loving pathologists, but get non-experts talking about science, medicine, and what happens in the lab – a critical part of promoting the profession.

References

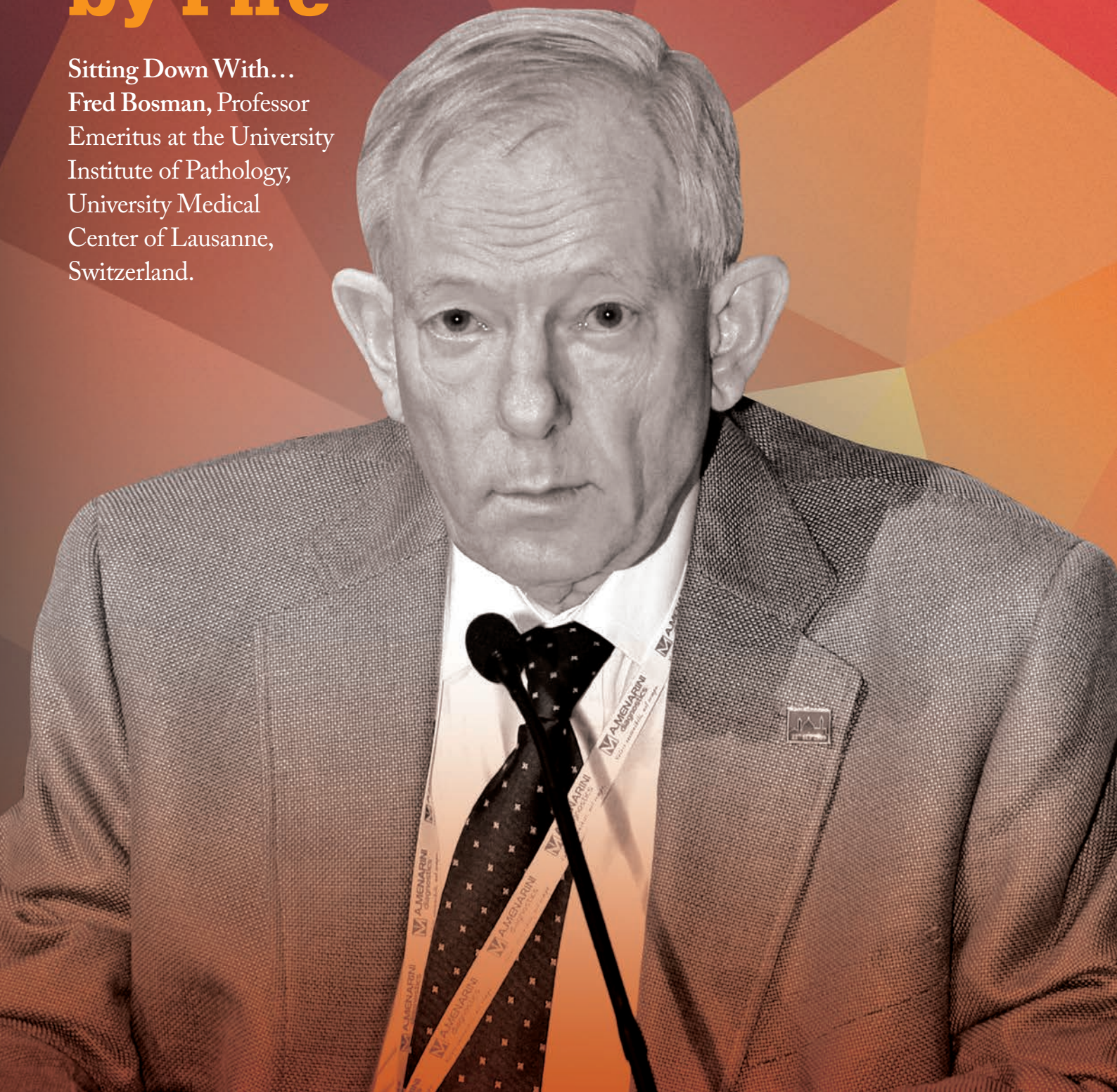
1. *S Jurkevica, "#PathArt: the art of tiny cells and tissues", (2015). Available at: http://bit.ly/1RM8PbY. Accessed January 13, 2016.*
2. *M Schubert, "The last respite of the socially inept?", The Pathologist, 3, 18–25 (2014). Available at: http://bit.ly/1Fs4FSH.*

Inverted embryonic mandible mimicking a muppet by @IHeartHisto.



Trial by Fire

Sitting Down With…
Fred Bosman, Professor Emeritus at the University Institute of Pathology, University Medical Center of Lausanne, Switzerland.



How did you find your way to pathology and tumor classification?

Initially, I wanted to become a surgeon, but after a brief stint in surgery, I realized I'd be bored pretty rapidly. Broadly speaking, I think you can classify physicians into those interested in curing disease and those interested in understanding it – and I realized that I was more interested in the latter. And by chance, when I decided that surgery wasn't the career for me, a position opened in pathology. I'd never thought about pathology, but it was love at first sight. I've never regretted it for one moment.

"It will impact the way every diagnostic pathologist works."

Tumor pathology has always been one of my major interests. The striking variety of different tumors is a big puzzle, and I've had a lifelong interest in walking the thin line between experimental pathology and diagnostic pathology to try to solve it. Recently, I've been lucky enough to get involved as a series editor of the World Health Organization's tumor classification series – one of the most influential things I've done, and a real pleasure. We're recording the transition from pure morphology to molecular classifications that impact the way patients are treated.

Pathologists have always looked at patterns of gene expression as they are reflected in morphology – but for a long time, we didn't know which genes were involved. When I became a pathologist, morphology was the endpoint of what we could contribute to disease diagnosis. Now that we have a better understanding of genetics, it's only the beginning. Morphology allows us to use microscopic tissue analysis to select which samples and tests to use, while molecular analysis allows us to understand tumor pathways and heterogeneity. So I don't think the morphological dimension of our work will disappear; rather, it has become the foundation upon which the molecular dimension is built. And that's not just something for a happy few; it will impact the way every diagnostic pathologist works.

Can you talk about your work in resource-poor communities? How have digital tools helped?

I've always perceived myself as a global citizen. Although I'm Dutch, I spent my boyhood in South Africa, which gave me a head start on feeling comfortable wherever I am in the world. In all of my leadership positions, I've actively looked for a partner institution in a resource-poor country to assist with pathology development. One reason for this is because the first position I held as a young pathologist was in Suriname, where I was confronted with a totally different pattern of disease and a significant lack of infrastructure. It was trial by fire! So I've spent my career trying to help others in similar positions – from Cuba to Cameroon.

Digital tools haven't changed the way pathologists look at tissue – but they have enormously facilitated things like training and long-distance consultation. At the moment, I spend about a month each year educating medical students in Nepal and supporting pathologists there. Virtual microscopy has allowed them to team up with expert pathologists all over the world much more easily. Pathology is pretty much the same everywhere, and Nepalese pathologists are well-trained. They're familiar with the sophisticated approaches of cutting-edge pathology, but they don't have the infrastructure to use many of them – not even ones we take for granted, like immunohistochemistry – so they lack the information we use to diagnose and classify disease.

What are the biggest challenges currently facing pathology?

Most pathologists are still mainly interested in what they can do with a microscope. But if the people involved in day-to-day diagnostics remain attached to their microscopes, they'll miss out on the molecular revolution. I think that's the main challenge in pathology right now: to reshape the discipline in such a way that pathology remains a key element in understanding disease, and goes beyond tissue samples to offer a molecular understanding of disease and therapy.

It's also important to get the public eye on pathology. It's not a very flashy discipline; a surgeon who has done some spectacular interventions with immediate success is much better perceived than a pathologist who is quietly sitting in his office, looking through a microscope. But pathologists can change that. We should be much more actively engaged in interaction with the public, teaching them who we are and showing them the importance of what we do.

What's the most interesting thing you've learned in your career?

The importance of education. This ties in with one of the main challenges in pathology, the lack of public awareness and the need to send the message that it's in an absolutely fascinating new phase of its development. The impact of a serious effort at any level of education, is absolutely crucial – and not only in terms of conveying factual information, but also in being a role model. It's not only about classroom teaching; it's a more holistic vision on the role of an educator. I've enjoyed research, but over time, I realized that the cited half-life of a good paper is only a few years. The half-life of a good educational effort is a generation – a pathologist's entire career.

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