Innovation in hematology: morphology and flow cytometry at the crossroads.

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In a rapidly changing world, the concept of innovation moves quickly from far fetched ideas, springing from a curious mind, to daily objects that nobody would ever dream of living without. One of the most obvious example is the smartphone, so much more than a telephone. It has, for instance, considering only the world of hematology, changed the mere concept of attending an international meeting. Gone are the days when we had to carry heavy books and frenetically search for that abstract which caught our eye late last night the paper of which is just about to be presented. Now, when the time arrives, the phone application pops up an alarm with the location, speaker, abstract, social network links, and even note-taking tools! And we take it all for granted!

Such exciting changes and ideas really do make themselves felt everywhere, even in such seasoned and validated methods as morphological and flow cytometry (FCM) analysis of pe-ripheral blood (PB) or bone marrow (BM). And if we go back to the smartphone, have you noticed how young (and not so young!) morphologists are swiftly replacing the now almost redundant CCD cameras and their sometimes complex software and share their doubts by directly capturing microscopy fields by placing the camera eye of their smartphone on the ocular of their microscope?

Indeed, laboratory diagnosis is rapidly changing from what it was in the past century, and this perspective essay will try and delineate where we are and where we are heading in these two fields, where "fast", "accurate" and "traceable" seem to be the keywords (…or perhaps these should be "faster", "more accurate" and "accreditable"?)

As reported in a collective European LeukemiaNet (ELN) review of the methodologies of leukemia diagnosis in 2015,[1] the future of morphology, although already moving forward in many places, still has digital wonders in store.[2] One of the pitfalls of such new approaches is the extent to which it still relies on the quality of smears and staining. However, huge progress has been made with more controlled slide dipping and reagent spray staining.[3] Although adjustments will still be necessary, it is to be expected that, by scanning large numbers of cells, robotized smearing and staining will allow image analysis systems to provide reproducible information.

The circulation of digitalized white blood cell (WBC) images for teaching or teleconsultation has long been in use.[1,4] But besides requiring time-consuming acquisition, it is eating-up large amounts of computer storage space. Increasingly in routine hematology, PB slide scanning and the generation of cell libraries is, however, gaining ground for computer-assisted differentials. It enables the storage of patient-related galleries of pre-analyzed and categorized single cells, allowing not only fast assessment but also quick retrieval of rare but crucial cells for concerted assessment (i.e. the elusive Auer rod-containing faggot-blast). Another advantage is it can provide documented/traceable information in a way that complies with the rules of accreditation. Yet, a limitation of these galleries is that, for any individual patient, at the moment, they do not allow storage and display of much more than the 100-200 cells manually reviewed in optical microscopy. Recent advances, such as whole slide imaging (WIS), mostly implemented by pathologists,[5,6] allow us to browse a whole sample and electronically store the location of cells of interest, instead of each identified image in a gallery. Given that enough storage space for WIS files is available, this technology could ultimately remove the need for those heavy slide cabinets with their smelly coverslip-mounted slides and old-looking numbered and dated labels, where unfailingly THE slide we are looking for has either disappeared or is the only one broken in its drawer. Instead, tomorrow's morphologists could open a given patient file and immediately get an instant self-generated gallery of cells on a specific slide retrieved from short files of cell co-ordinates automatically pre-identified by the expert system, while being allowed to take a closer look at whichever area the robot neglected, if needed. It is, however, expected that learning software, gathering knowledge with the accumulation of data in a Bayesian approach,[7] will be able to act as an intelligent expert system. Based on such information and thorough clinical data, further exploration and therapeutic options of a given patient will be more accurately prescribed by a more self-assured morphologist.

It is also likely that such expert systems will be able to concomitantly process morphology and FCM data, since, as detailed below, both methods produce results in the same time-frame, providing proper sampling has allowed both methods to be run. Of course, slide review of PB smears flagged during WBC assessment by cell analyzers is the most obvious of these combined approaches, since a liquid sample was, by definition, at the origin of the flag. It should, however, be noted that modern instruments increasingly combine the Coulter principle of impedance variation for cell counting and flow cytometry technologies, either on unstained cells or by taking advantage of auto-fluorescence or fluorescent staining of nucleic acids or proteins.[8] Currently used instruments already have so-called "research parameters" which store a large array of potential innovative applications for an early orientation of patients' clinical conditions.[9]

But let's go back to conventional differentials and we see that in FCM, the Hematoflow® solution,[10] with a simple and robust 6-antibody / 5-color combination already provides information on at least 13 leukocyte subsets. In the settings where it is used, it has drastically decreased the number of blood slides to be reviewed manually or digitalized down to about 4%.[10] One big advantage of FCM is that it can provide the higher precision of large numbers of counted cells,

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which, by the way, is also exploited in modern automated analyzers, in line with the increased accuracy predicted by the Rümke table.[11] Currently, solutions such as Hematoflow® or similar home-designed cocktails,[12] with fixed controlled panels and adapted software, already provide a large amount of information and modify the algorithms of further manual / digitalized slide examination, guiding the expert towards the anomaly under investigation and a quick diagnostic / therapeutic approach. It is to be expected that such applications will be developed into self-contained systems, where the primary tube will be directly processed for all FCM steps of sampling, antibody-staining, lysis-no-wash, histogram analy-sis and computer-assisted validation of normal samples or oriented flagging. Self-contained cost-effective solutions of this type could revolutionize the early steps of hematologic diagnosis in both malignant and non-malignant disorders.

Another impressive incursion of innovation in FCM is the appearance of LED-based multiparameter instruments using large panels well over currently established 8-10-color solutions. Such new generation instruments bypass the cumbersome step of compensations and are likely to allow much smaller amounts of both sample and antibodies to be used.

Another characteristic of PB that is often taken for granted, but not so well exploited, is that each microliter in an average sample of approximately10x10^9/L leukocytes contains one hundred more cells than those investigated in a blood smear. Yet flow cytometry can provide detailed and accurate information about each of these cells individually. Between 50 and 100 μL aliquotes are currently used, and this amount could easily be reduced for routine applica-tions, even allowing for a very poor sample of 1x10^9/L neutrophils, to assess 10^4 such cells in a 10 μL sample way beyond what even digitalized morphology can provide. In the future, the huge progress achieved in sophisticated automated flow paths for molecular assays of single-cell sequencing could be applied to the less demanding needs of morphology and FCM, circumventing the current limitations of manual pipetting, early syringe-based robots, and void volumes.

A lot of innovation has also already been put to work for electronically assisted investigation of red blood cells, i.e. stomatocytes, sickle cells, schiztocytes and Jolly bodies, both by smear digitalizing or use of developed research parameters of automated counters.[9,13,14] Platelets are also coming under increasing scrutiny.[9] Moreover, although at early stages, it is more than likely that combined morphology / FCM techniques will provide information on these cells. In fact, this is already the case in the above mentioned "research parameters" of last generation cell analyzers.[9]

One last issue where imagination and innovation still have to be put to the test is that of BM sampling. This still suffers from two major hindrances: it is highly operator-dependent and it is marred by the increasing need to perform more assays, i.e. morphology, flow cytometry, cytogenetics, molecular assays, thus requiring more volume. This results in hemodiluted samples that are responsible for variations between initial smears and further analyses in samples less and less representative of the patient's BM. Some solutions, however, seem to be gaining in maturity with training[15] and perhaps the development of assisted puncture devices, allowing for better reproducibility.[16] This would lead to huge progress being made also in the other fast-developing field of minimal residual disease, where there is still no real consensus as to the number of cells to be examined for accurate evaluation, while innovative solutions could be developed especially in lysis-no-wash procedures of BM samples.

In conclusion, while clearly being at the crossroads between classical and futuristic technologies, both morphology and FCM need to better integrate the results they can quickly provide at the bedside within efficient algorithms to refine diagnostic and therapeutic indications in the global aim of personalized medicine.

Even more far fetched, as mentioned at the beginning of this paper, the dream of non-traumatic *in vivo* cell counts, at least for basic parameters, is already becoming a reality.[8,17] But that's another story…

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