Digital holographic microscopy to identify cellular biomarkers of psychiatric disorders

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Quantitative phase-digital holographic microscopy offers high-throughput label-free detection capabilities for identifying cellular biomarkers of mental disorders.

Psychiatric disorders are a major public health concern. While the development of neuroleptics and antidepressants has led to significant improvements in patients’ conditions, available treatments remain largely insufficient and unsatisfactory.\(^1\)\(^2\) Advances in the field are hindered by a shortage of tools to conduct adequate biological measurements that can then guide diagnosis and treatment. This is particularly critical in the light of growing evidence that shows that early treatment of psychiatric disorders—already in their prodromal phase and before the onset of debilitating symptoms—leads to significantly improved clinical outcomes.\(^3\) Hence, there is a need to identify in children biomarkers or endophenotypes that reflect a high risk of later developing the disease. To date, however, there is insufficient data to support any of these biomarkers as vulnerability, diagnostic, or prognostic factors, notably regarding bipolar disorders.\(^4\)

The most relevant investigations are longitudinal studies conducted on cohorts of patients who have a specific psychiatric disorder, and on their children (high-risk children). In addition, psychiatric disorders are frequently comorbid with other mental illnesses and with endocrine, cardiovascular, and metabolic diseases (e.g., diabetes mellitus and obesity). These disorders must be considered as a multi-system condition, and their underlying neurobiological bases are complex. Currently, only studies that combine several biomarkers have been successful in providing high accuracy for the diagnosis of major depression, or for separating acute mood states (mania and depression) from controls.\(^5\)

Therefore, the identification of such reliable early biomarkers for vulnerability, neuroprogression, and diagnosis of mental disorders requires identification and longitudinal study of various sets of biomarkers that are very diverse. These include neuropsychological, neuroimaging (structural and functional MRI), and peripheral or cellular biomarkers. It is possible to study cellular biomarkers in somatic cells (fibroblasts) obtained by skin biopsy from patients and high-risk children.

To undertake biomarker studies, we have developed label-free quantitative phase-digital holographic microscopy (QP-DHM). This approach has high-throughput screening capacity and enables identification of novel cellular biomarkers thanks to its capacity to quantitatively and non-invasively measure a wealth of cell parameters, enhancing understanding of multifaceted cellular processes.\(^6\) QP-DHM provides real-time 3D images of transparent living cells, with an axial sensitivity of a few tens of nanometers, and without the use of any contrast agent.\(^7\)

The technique uses digitally recorded holograms that are interferometric in nature and are numerically reconstructed. Because these holograms enable correction of experimental noise,

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we can obtain a sub-wavelength measurement of the phase shift produced on the transmitted wavefront by the optically probed cells. This shift is the quantitative phase signal (QPS) (see Figure 1). The QPSs provide quantitative measurements of various biophysical cellular parameters in living cells. These include cellular surface, morphometry, absolute volume and volumetric changes, dry mass, membrane fluctuations at the nanoscale, and biomechanical properties, transmembrane water permeability, and current.\(^8\)

The hologram numerical reconstruction at the heart of QP-DHM enables autofocus without any mechanical action. Thus we can obtain an extended depth of focus. This numerical flexibility, combined with the high sensitivity and unique information about cell morphology and content that QPS provides, makes QP-DHM an appealing imaging modality. The method has the potential to perform original image-based screening aimed at discriminating a variety of cellular phenotypes, which is a prerequisite for efficient identification of new cellular biomarkers of disease.\(^9\)

We have demonstrated QP-DHM by measuring red blood cell (RBC) membrane fluctuations at the nanoscale, and the energy distribution of the fluctuations’ different vibrational eigenmodes.\(^10\) A study we are conducting in a cohort of diabetic patients shows a correlation between energy distribution among vibrational eigenmodes of RBC membranes and levels of glycosylated hemoglobin (see Figure 2).

In summary, the detection of multiple biophysical cell parameters scaled for a high-throughput cellular screening assay enables monitoring of several cellular processes concurrently. It also allows monitoring of modulations in these processes as a function of the transcriptome and metabolome of different cell types derived from controls, patients, and high-risk subjects. Thus, within the framework of longitudinal studies conducted on cohorts of patients and their children, our high-throughput non-invasive approach is highly promising for identifying new and original early cell biomarkers or endophenotypes of mental disorders. Our approach offers an invaluable tool for early diagnosis, improved stratification, and assessment of disease progression. It also enables monitoring of treatment outcome, which may be used for personalized medicine.\(^11\) Our future research will aim to identify high-risk cell biomarkers of major psychoses by performing QP-DHM multimodal studies of neuronal cells obtained from patients suffering from schizophrenia, bipolar, and major depressive disorders, as well as their high-risk offspring. Indeed, genetic reprogramming techniques (induced pluripotent stem cells, for example) enable the conversion of somatic cells collected from patients into neurons (grown in culture) that have the genetic makeup necessary for the development of certain phenotypes of the disease.

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