Reviewer's report

Title: Evaluation of FTIR Spectroscopy as a diagnostic tool for lung cancer using sputum

Version: 4 Date: 12 November 2010

Reviewer: Luca Quaroni

Reviewer's report:

Reviewer's report General Comments

The authors evaluate the viability of FTIR spectroscopy in discriminating between sputum samples from healthy controls, and patients affected by lung cancer. The work presents a comparison of the median FTIR spectra obtained from such samples and reports significant differences between healthy and affected individuals. Multivariate analysis is used to provide a quantitative discrimination between the samples. The authors propose that such capability for discrimination could provide the basis for a diagnostic method for rapid and non-invasive lung cancer detection.

The authors report appreciable spectral differences between healthy and affected individuals and this is by itself an interesting result and makes the work worth publishing. The work adds to the growing literature reporting infrared spectral changes of various pathologies. This work is a promising development towards the aim of providing a rapid and cost affective method for large-scale and frequent screening of high risk patients. Its impact could be considerable if the authors do follow up with the extension to larger datasets and the development of solid predictive models, as they promise in the conclusions.

One stumbling block that I see on the way towards this development is that FTIR diagnostic methods involving samples where the original tissue architecture has been lost (e.g. using exfoliated cells or tissue homogenates) have been controversial because the sample is representative of only a portion of the tissue and prone to contamination from other tissues. The current work is based on sputum-derived samples, which could also suffer from similar limitations. The authors have awareness of this issue and have commendably taken precautions to address this potential problem, such as separate histopathological examination to confirm bronchial origin of the samples. The results of these controls are encouraging and the samples appear to be representative of the pathology. Nonetheless the final assessment will come with the scaling up of sampling size, which is still modest in the present work. If results are consistent throughout large samples and there is no need of independent inspection by a pathologist to guarantee sample quality, this will indeed lead to a successful diagnostic method for high-throughput screening. The authors have all my encouragement and support for their future work in this direction.
Minor Essential Revisions

I have only one major revision to request at this point, which is actually related to more general suggestion made in the previous report, concerning the detailed discussion of DNA contribution to the spectra. The third paragraph of the discussion (“The 6 peaks described in Table I, etc.”) has some issues. It contains one wrong statement of molecular mechanics, namely “A rise in absorbance at a wavenumber in one sample relative to another is due to the increase in frequency of a bond vibration mode”. This is not generally true, although it happens to be for some specific chromophores and modes (the opposite happens as well). There is no proof that this does apply to the present situation and the statement should be avoided. For what concerns vibrational coupling between chromophores, proving it requires a combination of approaches, including independent structural measurements, measurements of polarized infrared absorption with coupled and uncoupled chromophores and quantum mechanical calculations. Obviously this is beyond the scope of this work and not relevant to it. In particular, an increase of absorbance in this typology of samples has been often shown to arise from more prosaic causes, such as difference in concentration of chromophores (as the authors themselves point out in a later sentence) and differences in cell size. Packing density of the chromophore has also been shown to affect its absorbance in a non linear way with respect to concentration (Wood, Biophys. J.; 2005). I must ask that vibrational coupling should not be represented as the only explanation for a difference in absorbance between two samples. All the other options mentioned above should be equally brought forward.

Minor issues not for publication

1. In “Methods” the acronym NSCLC appears for the first time in text of the revised version. Its definition is around the middle of “Discussion” and should be moved here.
2. In the “FTIR” subsection of “Methods” “compromised” should be replaced with “comprised”
3. In ”Discussion”, paragraph 3, “The 6 peaks …” should be typed as “The six peaks…”, if this sentence is retained after the correction requested above.