Novel approach to correlative imaging

by Dykas Michal, Correscopy

What is correlative imaging?

Increasing demand of high quality research forces research community to find new ways of obtaining data. Important part of the research is data acquisition from imaging devices. Imaging using various types of microscopy or spectroscopy is an essential technique used in many types of research in both academia and industry. Imaging of a single sample (or part of it) can be performed using number of techniques subsequently and the collected data can be compared and correlated, such imaging is named a correlative imaging. The correlative imaging, or correlative microscopy, was conventionally related strictly to correlation of the information acquired from two imaging devices about exactly the same location on a sample (e.g. a single cell), usually: Light Microscope (LM) and Scanning Electron Microscope (SEM) were used. To achieve such correlation manufacturers offer interchangeable sample holders or produce sophisticated all-in-one imaging devices. Correlative imaging techniques bring whole new range of possibilities in data analysis. Data correlation can be done using available software, resulting in the conclusions which can be made without a doubt. The correct data correlation is a factor that makes this techniques unique compared to the standard, uncorrelated imaging protocols.

What are the problems?

Availability of the solutions.

Imaging devices used for correlative imaging are sophisticated machines which require very high initial investments, on which most of the laboratories are not ready to take. This results in a very limited access to the correlative imaging technology. On the other side, few laboratories build their own custom made solutions which combine a few imaging techniques they are interested in, but these are very rare, difficult to make and often expensive. Also the available interchangeable sample holders require the laboratories to buy all facilities from the same manufacturer: either all new or as additional devices in the laboratory, greatly limiting the choice. Moreover, these manufacturers offer only a limited choice of the techniques which can be correlated as there is no single manufacturer which is an expert in all available techniques [1, 2]. Also all-in-one imaging devices often compromise on the performance compared to the individual devices, reducing the quality of the data.

Lack of data for correlation.

Even the data correlation software is accessible to the researchers, the problem lies in the data availability for its processing.

Go around solutions.

Some of the issues can be solved by using gridded coverslips which are often used in the light microscopy, mainly for biological applications. Thanks to the grid, one can navigate through the sample and find desired location. However, this solution have some drawbacks, like necessity of using a new substrate for experiment (gridded coverslip [3, 4]) which may affect behavior of the living sample (e.g. cells) and is only applicable for light microscopy on which the grid is visible. Some researchers prepare special marks on the surface by depositing e.g. metal structures or engraving the surface to help navigate through the sample. However, these solutions could affect the living samples - metal can influence the cellular processes, or engraving, changing a surface roughness, that can change cell adhesion to the surface - making the results doubtful.

There is a lack of commercial product which allows to localize exactly the same location of the sample on various imaging devices regardless of imaging technique and device manufacturer which would not have the described drawbacks.

Solution

An easily applicable device which could be used straightforward at various laboratories in many research fields would be a great solution to a problem.

- The device shall use the sample substrate which researcher is interested in, and it should not be imposed by the device.
- The substrate must have no additional marks, hence no influence on the sample is possible.
- Device will allow to collect the data from exactly the same location on the sample, which will be then used by software existing on the market for the data analysis and correlation.

Correscopy developed a device which allows to obtain desired data exactly from the same location of the sample for the data correlation (**figure 1**). The example of its application is shown in **table 1,** where four different techniques were used to image exactly the same cell. This set of the images provides large amount of the information which can be used to form a correct conclusion. It is worth mentioning that all these images were obtained from devices provided by various manufacturers as shown below. The user is not limited anymore to devices from single manufacturer. Almost any device available in user's or collaborators' laboratory can be freely used. As it can be observed the initial information about an interesting sample can be obtained on simple light microscope which is often available on the work bench while doing an experiment. If any interesting sample or

Table 1. Example of the imaging using the Correscopy setup on biological sample. Imaging was done using 4 different techniques provided by 4 different manufacturers. HeLa cell was cultured in presence of carbon nanotubes for 24h and then imaged by various characterization techniques. Received set of the data gives information about the cell morphology (LM, AFM and SEM), the distribution of the CNTs inside the cell (RS) and mechanical property if the cell (AFM). Scale bar 5 μm.

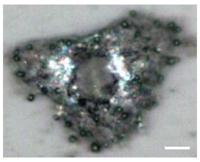
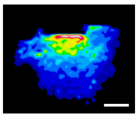
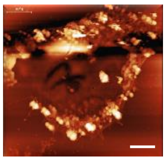
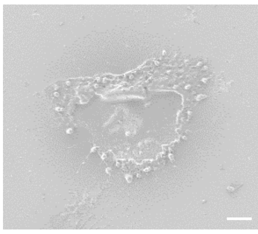
Technique used	**Results**	**Device manufacturer**
Light microscopy		 Nikon
Raman spectrosopy		 Horiba
Atomic force microscopy		 Bruker
Scanning electron microscopy		 Carl Zeiss

Figure 1. Correscopy setup which comprises multiple sample holders and adapters for various imaging devices supported by the software.

sample's area is identified, one can continue collecting higher quality images and different information from other devices, such as shown here: Raman Spectroscope (RS) which provides information about vibrational, rotational, and other low-frequency modes in an observed system; Atomic Force Microscope (AFM) which provides atomic resolution image of the surface, mechanical, electrical, magnetic, etc. information about the sample; Scanning Electron Microscopy which provides high-resolution image of a surface e.g. cell morphology. Such unique set of the data shows a great potential of the device developed by Correscopy. The other possible techniques which can be used are numerous and it mainly depends on the user's needs and access to the imaging devices. The other example, shown in **figure 2** below, presents two different sets of cells (fibroblasts) imaged by two super-resolution techniques: super-resolution LM and AFM for a direct comparison of the fluorescence data and mechanical properties of the single cell. Used imaging devices were already available at laboratory and no modifications to them were made. Cell imaging is the only one example of the application of this technology, there are many other possibilities, especially in biological research e.g. histology where the same part of the tissue can be examined on each device letting no mistake to be made in the conclusion.

Biological research is one of the possible applications of this technology. Any branch of research were imaging devices are used can greatly benefit from it: chemistry, material research, or engineering. Moreover, it also can be applied in commercial service imaging laboratories as the customer can exactly pin point which location of sample he is interested in and the service provider can image that particular location, creating win-win situation for both the sides.

Correscopy provides the device which is customized for each set of the imaging devices the laboratory uses. There are few minimal requirements from the device side. The device's sample stage[1] must be able to do X and Y transitions and a simple preview of a sample is required[2]. Provided "plug and play" solution does not require any physical modification of the microscope, hence guarantee will not be void or user does not need to obtain permission from a device owner.

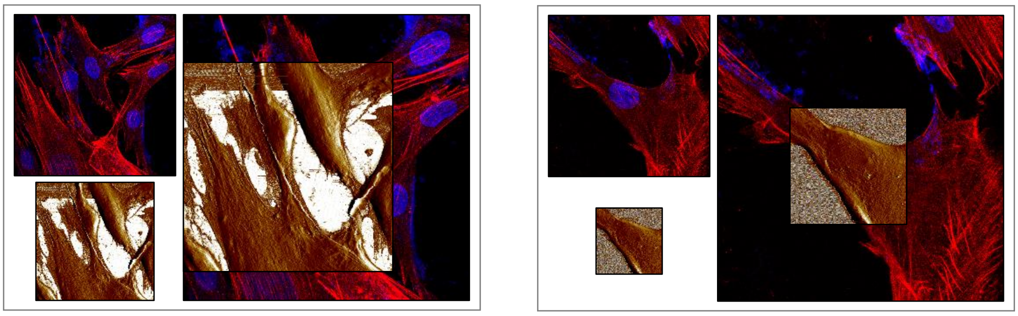


Figure 2. Fibroblasts imaged by two super-resolution techniques: LM (Carl Zeiss LSM 880 with Airyscan) and AFM (Bruker catalyst). The fluorescence data about the cell structure can be compared directly with the AFM results which provide the information about the mechanical properties of living cell. FOV for LM is 140μm and for AFM images 100 μm (left image) and 60 μm (right image).

[1] Sample stage can be provided on customer request.

[2] Please contact Correscopy for more information.

Conclusion

Correscopy provides a patented device that make possible to characterize exactly the same sample on any microscope or spectroscope even if it was not prepared for it by its manufacturer. The obtained data can be easily compared and correlated using any data analysis software. The solution provides undisputed data which makes clear-cut conclusions.

About the author

Michal Dykas is a founder of Correscopy and the inventor of the solution presented above. He has a PhD degree in integrative sciences and engineering with 5 years of experience in imaging using various techniques used mainly in biological research. Moreover he has engineering degree which helps him to design tailored solutions to the imaging challenges.

About the Correscopy

Correscopy is a start-up company solving real life problems regarded to correlative imaging. Correscopy provides easily available, missing link in the imaging process which allows the direct comparison of the results obtained from various imaging devices. Our mission is to improve quality of research by providing technology allowing correlation of the data from various imaging devices.

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