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 data acquisition. An important part of the research is data acquired 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 can be performed using a number of imaging 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 the correlation of the information acquired from two imaging devices, about exactly the same location on a sample (e.g. a single cell), usually: a Light Microscope (LM) and a Scanning Electron Microscope (SEM). To achieve such correlation manufacturers offer interchangeable sample holders or produce very sophisticated all-in-one imaging devices [1-8].

From the data obtained using correlative imaging techniques, many additional parameters of the sample can be discovered and calculated by using already available on the market image (data) correlation software - resulting in the conclusions which can be made without a doubt.

Where are the problems?

Availability of the solutions.

Imaging devices used for correlative imaging are often very sophisticated machines which require very high initial investments, on which most of the laboratories are not ready to take. This results in very limited access to the correlative imaging. Few laboratories build their own custom made solutions which combine few imaging techniques they are interested in, but these are very rare. Moreover, such all-in-one commercial imaging devices often compromise on the data quality compared to the designated devices, reducing the quality of the data. Also, the available interchangeable sample holders require the laboratories to buy specific additional microscopes, greatly limiting the choice. Moreover, these manufacturers offer only very limited choice of the techniques which can be correlated as there is no single manufacturer which is an expert in many techniques.

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.

Solution

There is a lack of commercial product which allows localizing 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.

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

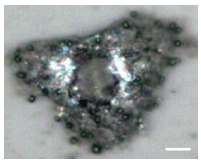
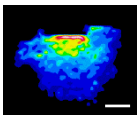
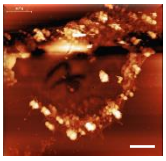
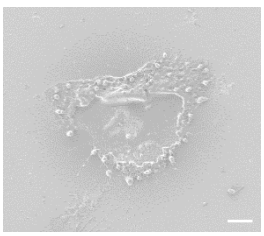
- The device must be applicable to the microscopes which are already available in the laboratory
- Device will allow collecting the data from exactly the same location on the sample, which will be then used by existing on the market software for the data analysis and correlation.

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

- The device shall use the sample substrate which researcher is familiar with, and it should not be imposed by the device.
- The substrate must have no additional marks, hence no influence on the sample is possible.

Correscopy developed a device which allows getting desired data from exactly the same location of the sample for the data correlation (**figure 1**). The example of the application is shown in **table 1** where four different techniques were used to image exactly the same cell. This set of the images provides a 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 single manufacturer devices. Almost any device available in one or collaborators laboratory can be freely used. As it can be observed the initial information about the interesting sample can be obtained on simple light microscope which is often available on the workbench while doing an experiment. If any interesting sample or 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 Microscope (SEM) which provides high-resolution image of a surface e.g. cell morphology. Such unique set of the data shows the great potential of the device

Table 1. Example of the imaging using the Correscopy device 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		 Carl Zeiss
Raman spectrosopy		 Horiba
Atomic force microscopy		 Bruker
Scanning electron microscopy		 Carl Zeiss

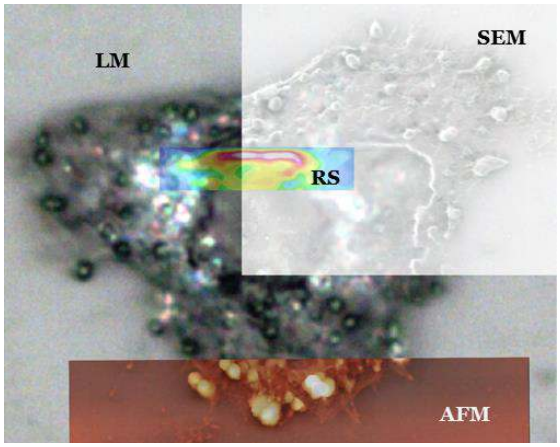


Figure 2. Example of the data representation. The data correlation can be done between the techniques researcher is interested in at the moment. Here SEM, AFM and RS techniques were correlated with light microscopy.

developed by Correscopy. The example of "all in one" figure correlated data is presented in **figure 2**. All the data is correlated to the LM image. Depending on the data correlation needs, a researcher can correlate the data freely with each other.

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 3** below, presents two different sets of cells (fibroblasts) imaged by two high-resolution techniques: 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 the laboratory and no modifications to them were made. Cell imaging is 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 only one of the possible research fields of this technology. In any field of research where imaging devices are used can greatly benefit from this technology: chemistry, material research, physics, and engineering. The example of material/physics research application is shown in **figure 4**, where quantum dots were imaged by two techniques: SEM and AFM. As can be seen, the feature size is in a range of nanometers, showing that any sample regardless of its size can be used for correlative microscopy using our technology. The actual accuracy of the technique can be compared with the accuracy of the microscope stage used. As long the sample is not fully homogeneous, the correlation can be easily performed. The other example of the non-biological application of RS–SEM correlative imagine can be seen by following this link: https://youtu.be/263fE47NHHE.

Moreover, this technology can be also applied in a commercial service imaging laboratories as the customer can exactly pin point which location of the sample he is interested in and the service provider can image that particular location, creating a 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

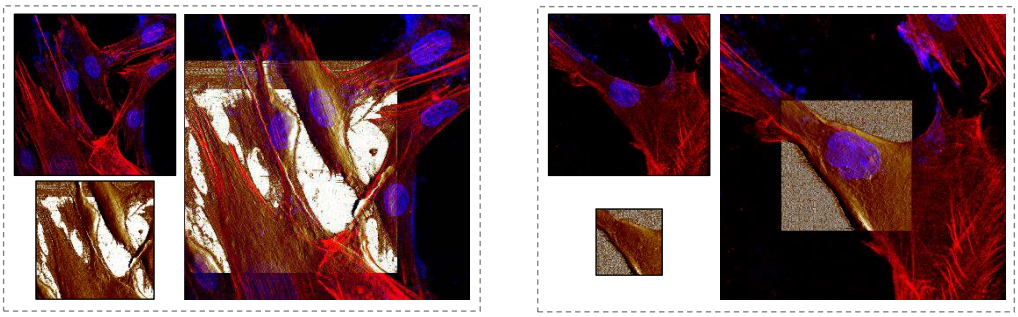


Figure 3. Fibroblasts imaged by 2 high-resolution techniques: LM (Carl Zeiss LSM 880 with Airyscan) and AFM (Bruker catalyst). The fluorescence data about the cell structure is correlated with the AFM results which provide the information about the mechanical properties of living cell. Direct correlation of cell structure and cell mechanical property was easily obtained. FOV for LM is 140µm and for AFM images 100 µm (left image) and 60 µm (right image).

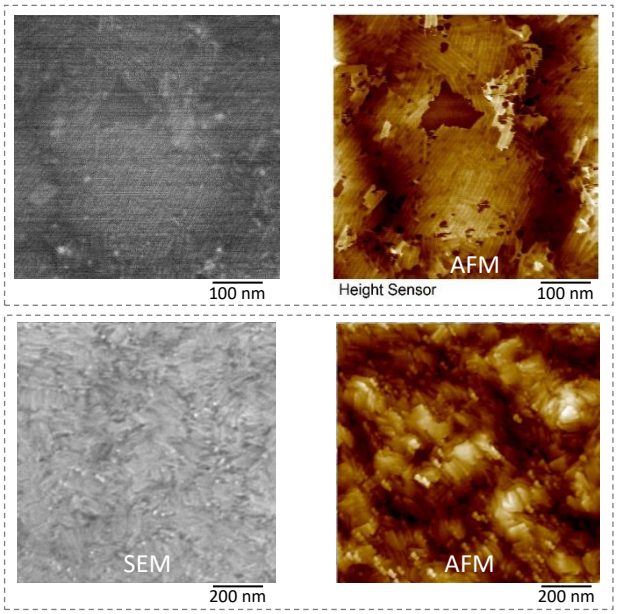


Figure 4. Quantum dots imaged by 2 techniques: SEM (FEI Quanta) and AFM (Bruker Icon).

sample stage[1] must be able to do X and Y transitions and a simple preview of a sample is required[2]. Provided solution is ready to be used on the imaging devices, it does not require any physical modification of the device, hence guarantee won't be void or a user doesn't need to obtain permission from a device owner. The wide list of microscopes and techniques, tested till now, is presented in an appendix. As can be seen, for our technology it does not matter if the microscope is a low, or high end, as well if it is old or brand new device, **it works for all**.

Conclusion

Correscopy provides a patented set of tools 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, the inventor of the solution presented in the article. He obtained the Ph.D. degree in integrative sciences and engineering with 5 years of experience in imaging using various techniques used in biological research, moreover, he has an engineering degree in automation and robotics which allows him to design tailored solutions to the imaging challenges.

About the Correscopy

Correscopy is a startup company solving problems regarded to correlative imaging. Correscopy provides the missing link in the imaging process which allows the direct comparison of the results obtained from various devices. Correscopy mission is to improve the quality of research by providing technology allowing direct correlation of the imaging data.

[1] automated or manual

[2] Please contact Correscopy if you need microscope verification

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List of tested techniques and microscopes:

		Type of microscopy	*Manufacturer*	*Microscope*
1	***1***	***Light microscopy***	**LEICA**	DVM6
2			**NICON**	Eclipse ME600
3				Eclipse Ni
4				Labophot-2 *(1980's)*
5			**MOTIC**	BA310
6				BA310MAT
7			**ZEISS**	Axio Lab.A1
8				Axio Observer Z1m
9				Axio Imager
10				Primo Star
11	***2***	***Fluorescent / Confocal Microscopy***	**LEICA**	DM 6000 CS
12				SP5
13			**OLYMPUS**	*IX 81*
14				*IX 71*
15			**ZEISS**	Axiovert
16				LSM 510
17				LSM 710
18				LSM 880 (+Airyscan)
19	***3***	***Scanning Electron Microscopy***	**COXEM**	*EM30 (desktop SEM)*
20			**FEI**	*Helios*
21				*Quanta FEG 250*
22				*Verios*
23			**HITACHI**	*SU8010*
24			**JEOL**	*JSM 5410 (1994)*
25				*JSM 6701F*
26			**TESCAN**	*MIRA*
27			**TESLA**	*BS 340 (1989)*
28			**ZEISS**	*Merlin*
29	***4***	***Infrared Spectroscopy***	**ANASYS INSTRUMENTS**	*nanoIR2*
30			**BRUKER**	*Hyperion 3000*
31			**THERMO SCIENTIFIC**	*Nicolet™ iN™10*
32				*Nicolet™ Continuum™*
33	***5***	***Raman Spectroscopy***	**HORIBA**	*HR*
34			**RENISHAW**	*inVia™ confocal Raman microscope*
35				*inVia™ µRaman (with Leica LM)*
36	***6***	***Atomic Force Microscopy***	**ASYLIUM RESEARCH**	*MFP 3D BIO*
37			**BRUKER**	*Catalyst*
38				*Fastscan*
39				*Icon*
40			**NANOSURF**	*Easyscan 2*
41	***7***	***X-ray photoelectron spectroscopy***	**KRATOS**	*Supra*
42	***8***	***Secondary-ion mass spectrometry***	**ION-TOF**	*TOF.SIMS 5*
43	***9***	***Nanoindentation***	**CSM**	*nanoindenter*
44	***10***	***Correlative microscopy***	**WITEC**	Alpha