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1 D6.1: Design of multimodal characterisation system(s) that incorporate complementary techniques relevant for carbon-based nanocomposites research

Abstract: Achieving a thorough understanding of the properties and function of carbon-based nanocomposites (CNC), and of their interactions with other materials or with biological species, requires a concerted used of complementary investigations techniques. Implementing correlative imaging assays with independent characterization instruments is however very difficult, and in some cases even impossible. There is thus great need for the advent of multimodal systems specialized for studying complex, and interdependent properties of CNCs. While a system comprising every possible technique that is useful for resolving various properties of CNCs is of course impossible to achieve, many investigations techniques share significant architectural similarities, which can be exploited to design and develop multimodal systems that harbour a variety of techniques with constructional overlap but with highly distinct, yet complementary, contrast mechanisms. Furthermore, such multimodal systems can be coupled together, to result in even more complex architectures, providing access to a wide range of physico-chemical properties of CNCs. Here we propose the potential design of such a multimodal system, which we envision to incorporate near-field and far-field optical investigation techniques, augmented by scanning probe microscopies, all being highly complementary in terms of contrast mechanisms, accessible CNC properties, scales, and in many other regards. This design is meant to inspire groups working in high-resolution imaging to devote efforts to meeting an urgent need of the communities concerned with CNCs research (synthesis and functionalization), consisting in multimodal imaging systems that could enable next-gen characterization frameworks, allowing a better understanding of many properties and functionalities of CNCs still waiting to be resolved.

Credits:

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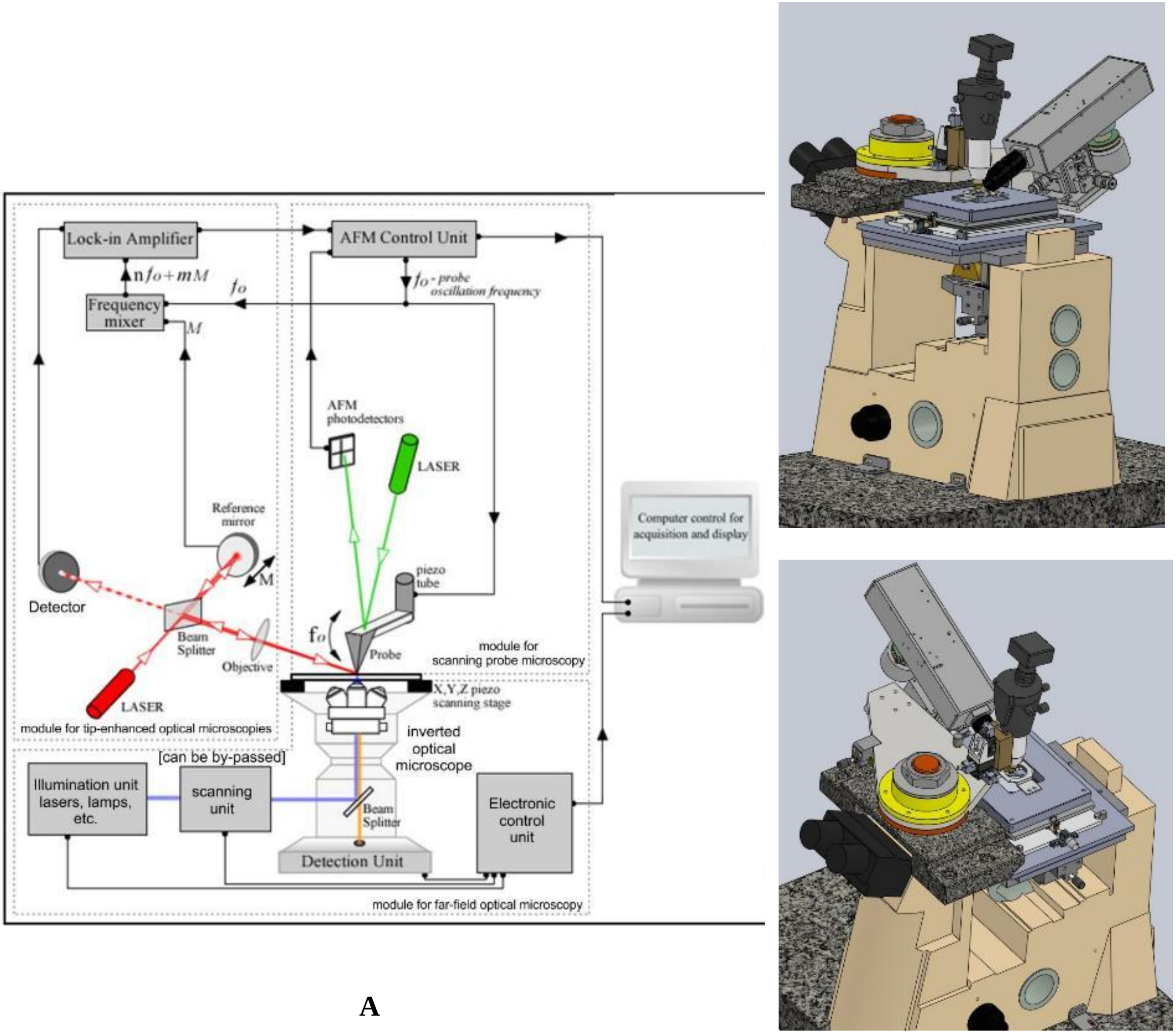
Motivation: The specifics of an investigation technique can be suitable to image specific structural, physical or chemical aspects, yet may exclude others(Caplan, Niethammer et al. 2011, Jahn, Barton et al. 2012, Smith 2012). In the context of high-resolution optical imaging, micro- and nano-scale data, as well as far-field and near-field data, complement each other and their consistent correlation can enable novel perspectives and open new research avenues in many critical research fields, such as biology, medicine, material science or device characterization. This statement can be straightforwardly extrapolated to non-optical techniques, such as scanning probe or electron microscopy, where the availability of complementary information at different scales provided by distinct imaging instruments is acknowledged as being highly important for achieving a thorough understanding of the studied sample. Correlative imaging can thus be regarded as the holy grail of modern characterization protocols, given that different physical, chemical and morphological properties of a sample of interest are bound to one another, and being able to visualize these all at once, and to understand the relationships that occur between these, is crucial for the correct understanding of high and ultra-high resolution optical and non-optical data (Caplan, Niethammer et al. 2011, Leslie 2011, Smith 2012, Walter, Paul-Gilloteaux et al. 2020). Such approaches are, however, often challenging. Investigating corresponding sample regions using different imaging systems often proves to be a cumbersome task, as the collected data sets need to be precisely matched and overlain. Identifying sample regions of interest after switching between imaging systems based on different contrast mechanisms, and working at different resolution scales, is time demanding (and sometimes impossible). These challenges can be addressed through the use of multimodal imaging systems that incorporate complementary techniques. While many leading companies in the instrumentation field already hold multimodal systems in their portfolio of products, which in most cases incorporate sets of techniques that exhibit similar architectures, at present no multimodal imaging system specifically designed for research on carbon-based nanocomposites (CNCs) exists.

The purpose of this deliverable is thus to propose a flexible design of a multimodal system incorporating complementary techniques relevant for carbon-based nanocomposites research, which may serve as a starting point for the implementation of such systems in the future. This design is intended to accommodate the implementation of mature, emerging, and forthcoming microscopy techniques useful to provide various types of information on carbon-based nanocomposites, and on their integration and interaction with other materials and biological species, such as proteins, viruses, cells, tissues, etc. Furthermore, the intention of ESSENCE is to design a system that is feasible to be implemented as an integrated/compact table-top prototype offering micro and nanoscale resolutions based on complementary contrast mechanisms, which collectively can shed light on aspects that cannot be resolved by a single technique. Such a system would fill the current market gap for multimodal imaging systems designed specifically for the characterization of CNCs and their interplay with other materials and biological items of interest.

Solution: The proposed design relies on a “sandwich”-type architecture, consisting of two or more interlinked modules that can probe co-localized regions of a specimen positioned in-between(Harke, Chacko et al. 2012, De Boer, Hoogenboom et al. 2015, Stanciu, Tranca et al. 2016, Staunton, Doss et al. 2016, Zhou, Cai et al. 2017, Cosentino, Canale et al. 2019). It builds on the expertise of ESSENCE consortium members preoccupied with high-resolution imaging and exploits past imaging architectures reported by them(Stanciu, Tranca et al. 2016, Stanciu, Tranca et al. 2017), together with current ongoing efforts in which they are engaged. In the diagram presented in **Fig. 1** we present a feasible architecture to incorporate optical techniques, scanning probe techniques, and hybrid scanning probe-light techniques, the latter exploiting the interaction of a sharp tip with focused light. In this configuration non-optical scanning probe techniques, together with the hybrid techniques building on tip-enhanced optical effects, are available in a module positioned above the sample plane, whereas far-field optical techniques, either widefield or scanning based, are available with a module positioned underneath the sample plane. Upon availability of transparent substrates, the CNCs can be probed in correlative sessions with both top and bottom imaging modules. The top module for scanning probe and tip-enhanced microscopy can be positioned either on a sliding rail or on a rotating swivel, so that other imaging modules can replace it, for example modules for scanning electron microscopy(De Boer, Hoogenboom et al. 2015, Hauser, Wojcik et al. 2017).



A

B

Fig. 1 Schematic diagram (A) and 3D model (B) of a multimodal system that can accommodate complementary optical, scanning probe, and hybrid imaging techniques, the latter exploiting the interaction of sharp tips specific to scanning probe microscopy with focused light. The sample is placed in the middle, and it can be accessed by scanning probe microscopy techniques, by means of an imaging module positioned above it. The same module for scanning probe microscopy can be augmented by an optical module that can probe the near-field of the sample, to extract nanoscale information provided by imaging techniques building on tip-enhanced effects or phenomena specific to tip-light interactions. This design is inspired by previous work of UPB (SG Stanciu et al., Biomed. Opt. Express, 2017), who reported a system for correlative imaging with confocal laser scanning microscopy and scattering-type scanning near-field optical microscopy and can accommodate a wide palette of additional techniques sharing architectural similarities. The 3D model depicted in B) illustrates the architecture concept, top: lateral view, bottom: bird's-eye view.

The scanning probe and tip-enhanced microscopy modules shown in **Fig. 1**, can be multiplexed to enable multiple scanning probe and tip-enhanced microscopy variants, as shown in **Fig. 2A)**. With detectors such as avalanche photodiodes or photomultipliers these can enable imaging of various tip-enhanced optical effects, while with spectrophotometers spectroscopic investigations of various optical properties can be enabled, **Table 1**. Such detectors can be either fibre-coupled, with the appropriate optics, or can be operated by free space. Other techniques that exploit the interaction of sharp scanning probe with focused light, and extract the information of interest from the deflection of the probe, can be enabled by specialized electronics coupled for the feedback mechanism of the scanning probe system, and to the quadrant photodetector of the light lever used for scanning probe microscopies (**Table 1**). The bottom module can rely on an inverted optical microscope base, equipped with a module for laser beam scanning, which is required for laser scanning microscopies. For such techniques, image acquisition is usually performed point-by-point, thus photodetectors such as photomultipliers or avalanche photodiodes are required. This bottom module can also be configured for wide-field microscopy, where the illumination is accomplished for the whole sample plane of interest at once and the image is recorded in a single snap-shot with a standard or scientific grade CCD/CMOS camera. Such configurations can be implemented to reversibly replace the laser scanning microscopy unit, as illustrated in **Fig. 1**, or can be implemented on a different light path for optical microscope stands with multiple lateral ports. Similar to the top modules for tip-enhanced optical imaging and scanning probe microscopy, the bottom module for far-field optical imaging can be multiplexed to incorporate a wide variety of optical techniques sharing common principles. In **Fig. 2B)**, we provide a potential scheme for a multimodal imaging module incorporating several far-field modalities operating either in transmission or in an epi-configuration (for the transmission configuration, the top module for tip-enhanced optical imaging and scanning probe microscopy must allow the positioning of a far-field detector). Depending on their specifics, these techniques can operate based on continuous-wave (CW) or pulsed laser illumination and can benefit from the contribution of optical devices that extend the wavelength ranges of the laser sources, such as optical parametric oscillators or photonic crystal fibers for white light generation. Furthermore, as shown in **Fig.2B)**, this design can accommodate special optics such as Vortex Phase Plates (VPP), which can be used for beam shape manipulation, e.g., creation of doughnut-shaped beams, required for pump-and-probe techniques inspired from Stimulated Emission Depletion Microscopy(Hell and Wichmann 1994) concepts, or more complex optical devices such as optical delay lines that provide possibilities for light pulse manipulation, which is also known to be of great benefit for pump and probe techniques.

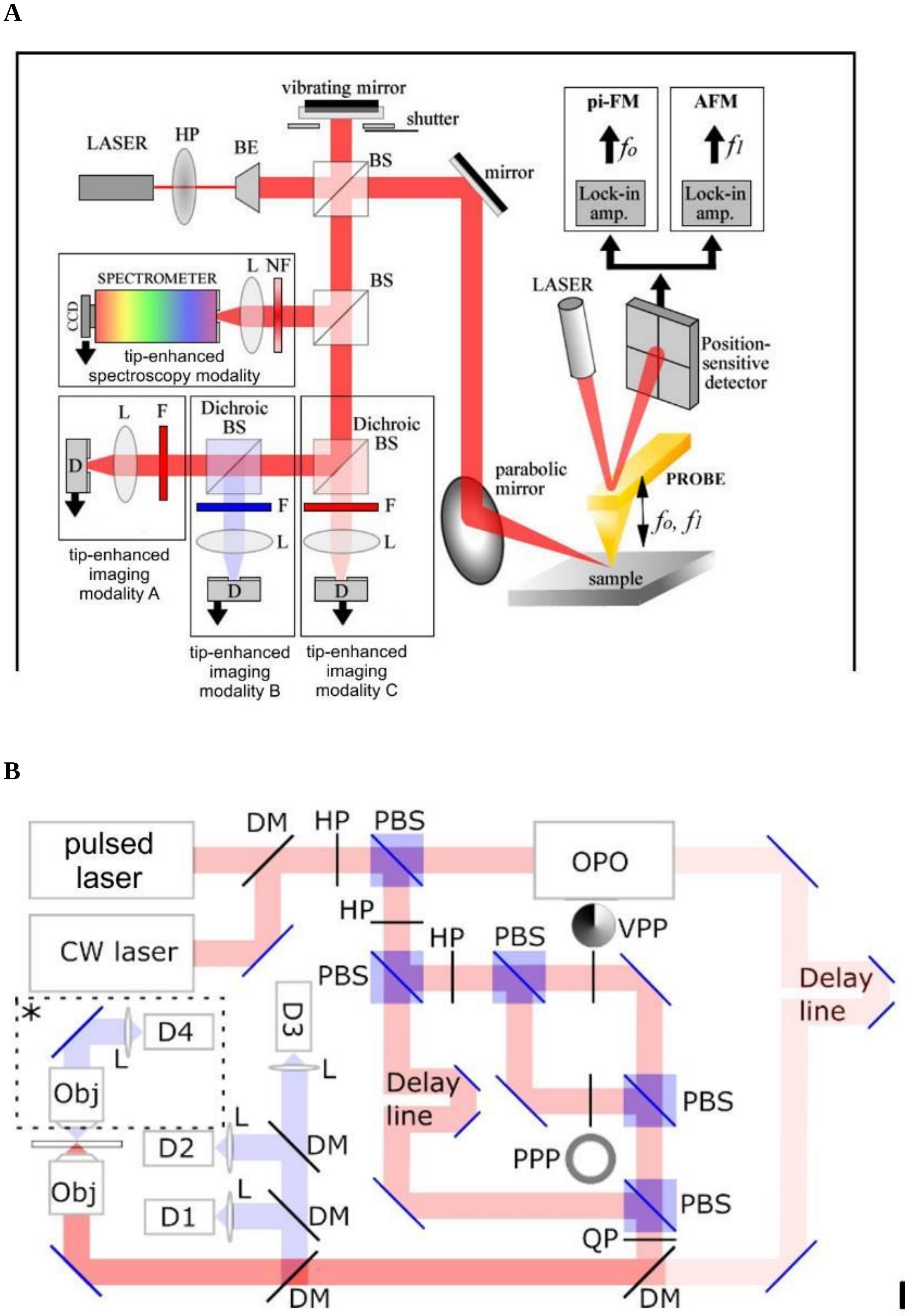


Fig. 2 a) Design concepts for multimodal imaging with scanning probe, tip-enhanced optical microscopy, and far-field optical microscopy techniques. A) Design concept of an imaging module for multimodal tip-enhanced optical microscopies, augmenting a module for scanning probe microscopy (HP: half-wave plate, BE: beam expander, BS: beam splitter, NF: notch filter, L: lens, DM: dichroic mirror, F: filter, D: detector). Detectors for three imaging modes and for one spectroscopic workmode are illustrated, but this design can be scaled up or down, depending on needs. A vibrating mirror can be used for signal modulation schemes, e.g., pseudo-heterodyne detection, to enhance signal-to-noise ratio of the detected signals. The light-lever, consisting of laser source, cantilever, quadrant photodiode, enables integration with scanning probe microscopy variants, and with techniques based on photo-induced force. B) Design concept of the imaging

module for multimodal far-field optical microscopies based on laser scanning (PBS: polarizing beam splitter, PPP: concentric π-shift phase plate for 3D resolution enhancement, DM-dichroic mirror; L-lens; PBS-polarizing beam splitter, D-Detector; Obj-Objective). Detectors for three imaging modes operating in an epi-configuration and for one workmode in transmission are illustrated, but this design can be scaled up or down, depending on needs. Beam shaping elements such as the VPP, or devices for temporal (Optical delay lines) or spectral (OPO-Optical parametric oscillator) manipulation can enable diverse complementary far-field microscopy work modes, performing in either scanning or widefield configurations.

In the next table, we present a series of optical, scanning probe, and hybrid techniques relevant for CNC research that can be implemented in a multimodal system featuring the designs presented in **Fig. 1** and **Fig. 2**. This list does not exclude the potential compatibility of other already existing or future imaging techniques. We provide as well references to relevant works that have previously employed the nominated techniques for studying important properties of various carbon-based nanomaterials.

Table 1: Imaging techniques relevant for CNCs research.

Technique name	Type	Resolution	Examples on relevance for CNC research
Fluorescence Confocal Laser Scanning Microscopy	Optical - far-field	>200nm	(Hazani, Naaman et al. 2003, Peng, Hu et al. 2010, Wang, Cao et al. 2010, Zhao, Rondin et al. 2017, Patel, Singh et al. 2019)
Reflectance Confocal Laser Scanning Microscopy	Optical - far-field	>200nm	(Porter, Gass et al. 2007, Piwko, Althues et al. 2015, Panchal, Yang et al. 2018, Chan, Chen et al. 2021)
Confocal Rayleigh scattering microscopy	Optical - far-field	>200nm	(Casiraghi, Hartschuh et al. 2007)
Confocal Raman Microscopy	Optical - far-field	>200nm	(Mews, Koberling et al. 2000, Balasubramanian, Friedrich et al. 2003, Kang, Nguyen et al. 2012, Girish, Sasidharan et al. 2013, Shojaee, Zandiatashbar et al. 2013)
Two-Photon Excited Fluorescence Microscopy	Optical - far-field	>200nm	(Li, Bao et al. 2012, Liu, Guo et al. 2013, Pramanik, Chavva et al. 2014, Yi, Yang et al. 2014, Singh, Sreedharan et al. 2019)
Second Harmonic Generation Microscopy (in scanning and wide-field configurations)	Optical - far-field	>200nm	(Slepyan, Maksimenko et al. 1999, An, Rowe et al. 2014, Shan, Li et al. 2018, Stepanov, Semin et al. 2020, Yang, Song et al. 2020)
Third Harmonic Generation Microscopy (in			(Stanciu, Ehlich et al. 2002, De Dominicis, Botti et al. 2004, Kumar, Kumar et al. 2013,

scanning and wide-field configurations)			Attaccalite, Cannuccia et al. 2017, Ha, Park et al. 2021)
Coherent Anti-Stokes Raman Microcopy	Optical - far-field	>200nm	(Galloway, Lewis et al. 2010, Duarte, Rehbinder et al. 2013, Goodhead, Moger et al. 2015, Paddubskaya, Dementjev et al. 2018, Paddubskaya, Rutkauskas et al. 2020)
Sum-Frequency Generation Microscopy	Optical - far-field	>200nm	(Zhang, de Aguiar et al. 2020, Hong, He et al. 2022, Zhou, Ge et al. 2022)
Stimulated Emission Depletion Microscopy	Optical - far-field	>20nm	(Tzeng, Faklaris et al. 2011, Stöhr, Kolesov et al. 2012, Wei and Min 2012, Arroyo-Camejo, Adam et al. 2013, Leménager, De Luca et al. 2014, Li, Ye et al. 2019)
Transient Absorption Microscopy	Optical - far-field	>200nm	(Huang, Hartland et al. 2010, Jung, Slipchenko et al. 2010, Gao, Hartland et al. 2011, Li, Zhang et al. 2015, Davydova, de la Cadena et al. 2016)
Saturated Transient Absorption Microscopy	Optical - far-field	>50nm	(Liu, Kumbham et al. 2016, Zanini, Korobchevskaya et al. 2019, Bi, Yang et al. 2020)
Light Sheet Microscopy	Optical - far-field	>200nm	(Huang, Zhu et al. 2019)
Stochastic Optical Reconstruction Microscopy	Optical - far-field	>20nm	(Li, Georgiades et al. 2018, Moon, Li et al. 2020, Scalisi, Pennacchietti et al. 2021, Nandi, Caicedo et al. 2022)
Photo-activated Localization Microscopy	Optical - far-field	>20nm	(Lu, Zong et al. 2021, Li, Kaminski Schierle et al. 2022)
Structured Illumination Microscopy	Optical - far-field	>100nm	(Chen, Dong et al. 2017, Teodori, Crupi et al. 2017, Singh, Sreedharan et al. 2019)
Tip-enhanced fluorescence microscopy	tip-enhanced optical effects (near-field)	>10nm	(Huang, Festy et al. 2005, Ostrowski, Nordmeyer et al. 2015, Kumar, Kalirai et al. 2019)

Tip-enhanced Second Harmonic Generation Microscopy	tip-enhanced optical effects (near-field)	>10nm	(Smolyaninov, Zayats et al. 1997, Dean and van Driel 2009, Shan, Li et al. 2018, Yang, Song et al. 2020, Zhou, Guo et al. 2022)
Tip-enhanced Raman Spectroscopy and Microscopy	tip-enhanced optical effects (near-field)	>1nm	(Hayazawa, Yano et al. 2003, Hartschuh, Qian et al. 2009, Ghislandi, Hoffmann et al. 2012, Beams 2018, Shao and Zenobi 2019)
Scattering -type scanning near-field optical microscopy	tip-enhanced optical effects (near-field)	>5nm	(Fei, Rodin et al. 2012, Shi, Hong et al. 2015, Hu, Luan et al. 2017, Xu, Ma et al. 2017, Tian, Chen et al. 2021, Wirth, Linnenbank et al. 2021, Zhang, Luo et al. 2022)
Tip-enhanced photoluminescence	tip-enhanced optical effects (near-field)	>1nm	(Chien, Li et al. 2012, Cao, Meziani et al. 2013, Lingam, Podila et al. 2013, Yang, Chen et al. 2020)
Photo-induced force microscopy	tip-enhanced optical effects (near-field)	>10nm	(Liu, Park et al. 2018, Kim, Khan et al. 2020)
Photothermal Force Microscopy / AFM-IR	tip-enhanced optical effects (near-field)	>10nm	(Bartlam, Morsch et al. 2018, Liu, Nørgaard et al. 2018, Mikhalchan, Tay et al. 2020, Yang, Lin et al. 2020, Menges, Yang et al. 2021)
Atomic Force microscopy	Scanning probe	>1nm	(Postma, Sellmeijer et al. 2000, Volodin, Ahlskog et al. 2000, Nemes-Incze, Osváth et al. 2008, Schniepp, Kudin et al. 2008, Liu, Ng et al. 2010)

Magnetic Force Microscopy	Scanning probe	>1nm	(Hong, Bekyarova et al. 2012, Rao, Matte et al. 2012, Li, Qi et al. 2013, Chuang, Matsunaga et al. 2021)
Electrostatic Force Microscopy	Scanning probe	>1nm	(Gil, De Pablo et al. 2002, Jespersen and Nygård 2005, Zdrojek, Mélin et al. 2006, Jespersen and Nygård 2007, Moser, Verdaguer et al. 2008, Burnett, Yakimova et al. 2011, Cadena, Misiego et al. 2013, Yalcin, Galande et al. 2015)
Force Modulation Microscopy	Scanning probe	>1nm	(Volodin, Ahlskog et al. 2000, Robinson, Rabot et al. 2014, Abooalizadeh, Sudak et al. 2019)
Kelvin Probe Microscopy	Scanning probe	>1nm	(Cui, Freitag et al. 2003, Liu and Li 2010, Curtin, Fuhrer et al. 2011, Kehayias, MacNaughton et al. 2013, Pearce, Eriksson et al. 2013, Yu, Giridharagopal et al. 2021)
Chemical Force Microscopy	Scanning probe	>1nm	(Yang, Zhang et al. 2002, Poggi, Lillehei et al. 2005, Hernandez, Bennett et al. 2013, Wang, Thong et al. 2016)

Conclusions: One of the most important bottlenecks that carbon research faces is the lack of specialized multimodal systems that can provide in-tandem access to highly complementary information on the complex physics and chemistry of CNCs, and on their interaction with other materials and with biological species. Here, we propose a proof-of-concept design, based on past expertise of ESSENCE members in multimodal imaging, that can be tuned to accommodate a wide variety far-field and near-field optical techniques, working at different scales, whose usefulness is augmented by availability of scanning probe techniques, available in the same architecture. We hope that this schematic design, together with the discussions that we carry, will inspire the advent and future development of multimodal systems specialized for CNC research.

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