

# Correlative Microscopy: Techniques, Triumphs and Opportunities

C. Patrick Royall<sup>1,2</sup>

<sup>1</sup>*Gulliver UMR CNRS 7083, ESPCI Paris, Université PSL, 75005 Paris, France.*

<sup>2</sup>*Physics Department, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK.<sup>a)</sup>*

**Summary.** Correlative Microscopy in its strictest sense, refers to the simultaneous imaging of the *same* region of a specimen by two or more distinct imaging techniques. Like many forms of microscopy, it had its origins in biological applications with imaging of the same region of a specimen using fluorescence microscopy and TEM<sup>1</sup>. This review will detail the imaging methods used and will provide examples of key insights gained from the combination of multiple imaging techniques. The applicability of the different techniques to a range of relevant samples is discussed, and also the extent to which different techniques are mutually compatible. We will also discuss the benefits of the more general case of using multiple imaging techniques *without* the requirement that the same sample region is imaged.

## I. BRIEF OVERVIEW OF MICROSCOPY TECHNIQUES RELEVANT TO PARTICULATE SYSTEMS

In the spirit of producing a self-contained document, that can be read without the need to endlessly refer to other sources, we aim to provide enough information for the reader to appreciate the contribution that each technique *by itself* can make. We presume that the while the reader is likely expert in a subset of the relevant techniques, that they are unlikely to be expert in all. We begin with Fig. 1 which indicates the lengthscales that each technique is appropriate to.

### A. Optical Techniques

We will include a discussion of Fluorescence Microscopy, Confocal Microscopy, Reflection Interference Contrast microscopy<sup>2</sup>. Super-resolution “nanoscopy” techniques such as photoactivated localization microscopy (PALM)<sup>3</sup>, STochastic Optical Reconstruction Microscopy (STORM)<sup>3</sup> and STimulated Emission Depletion STED<sup>4,5</sup>. In the context of correlative microscopy, it is worth noting that Fluorescence Microscopy typically considers optically thin specimens, while for example confocal and STED are bona-fide 3d techniques.

### B. Electron Microscopy Techniques

Brief description of TEM, SEM, Environmental SEM. EDX. We will outline new advances in *Cryo-EM*<sup>6</sup>.

### C. Atomic Force Microscopy

Explanation of principle AFM contrast techniques and what they reveal about the system: contact mode, tapping mode.

### D. Tomography

We will outline the use of tomography in characterizing particulate systems. For example, electron tomography to reveal the locations of nanoparticles in 3d<sup>7</sup>. We will also discuss focussed ion beam (FIB) milling and FIB-SEM 3d imaging<sup>3</sup>.

### E. Raman Spectroscopy

Brief overview of the Raman technique and how it may be applied to soft matter/fine particles<sup>3</sup>.

### F. Image Analysis

We will mention traditional methods such as image restoration via deconvolution<sup>8</sup>. Recently, AI and machine-learning methods have begun to be used to enhance the results of previous analysis methods<sup>9</sup>. We will also cover AI-assisted annotated segmentation and deep learning assisted image reconstruction.

### G. Discussion of strengths and weaknesses of each method

This will take the form of a “box”. The comparative resolution of each technique will be discussed, along with the nature of samples that are appropriate to it. Sample degradation mechanisms (eg beam damage, photobleaching) will be discussed a means towards an understanding of samples that may be appropriate for *multiple* imaging techniques.

Suitable properties of a sample (eg transparency for confocal microscopy), volatility relating to vacuum techniques will be discussed. We envisage a “box” which covers relevant systems of interest and their applicability for the various techniques considered here.

---

<sup>a)</sup>Electronic mail: [paddy.royall@espci.psl.eu](mailto:paddy.royall@espci.psl.eu)

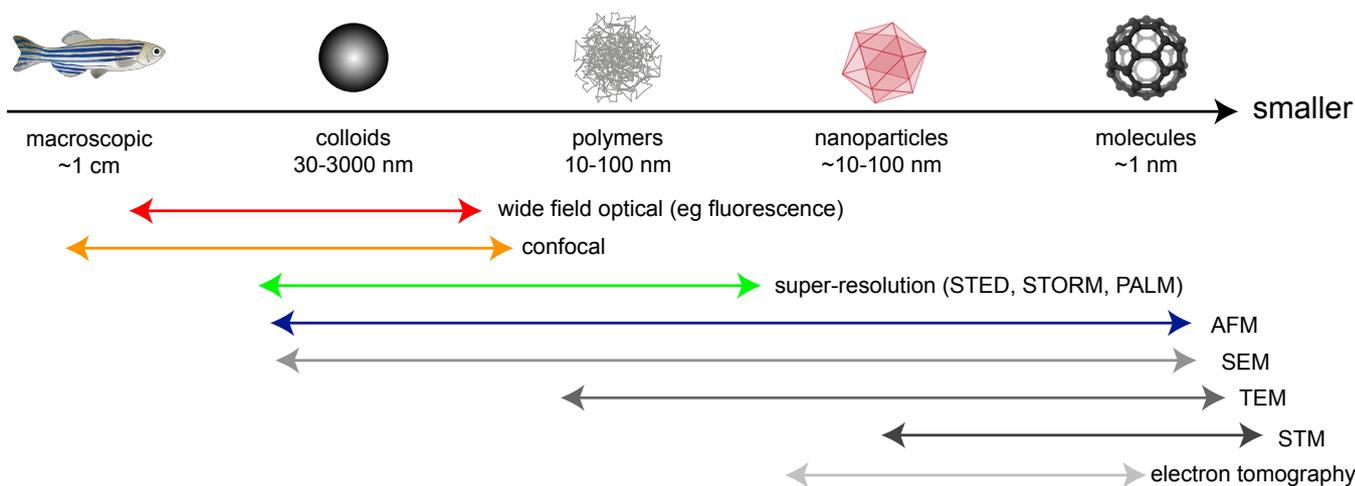


FIG. 1. **Lengthscales relevant to correlative microscopy.** Schematic indication of typical lengthscales of systems of interest. Approximate lengthscale regime for a selection of microscopy techniques. The lower bound is set by the resolution of the technique. The upper bound is set by practicability of imaging time and the instrument set-up.

## II. PUTTING IT ALL TOGETHER: ANALYSIS OF THE SAME REGION WITH MULTIPLE TECHNIQUES (CORRELATIVE MICROSCOPY)

### A. Integrated Light and Electron Microscopy Techniques

This is the first<sup>1</sup> and perhaps the most obvious use of correlative microscopy. See Fig. 2 for a schematic. In particular, one seeks to combine the nanometer resolution of electron microscopy with optical techniques, which may be more sensitive to chemistry or label specific information. An important part of correlating the differences in information supplied by the imaging techniques lies in bridging the different resolution of optical and electron-based microscopies<sup>3</sup>. Two flavours are possible – correlative light and electron microscopy

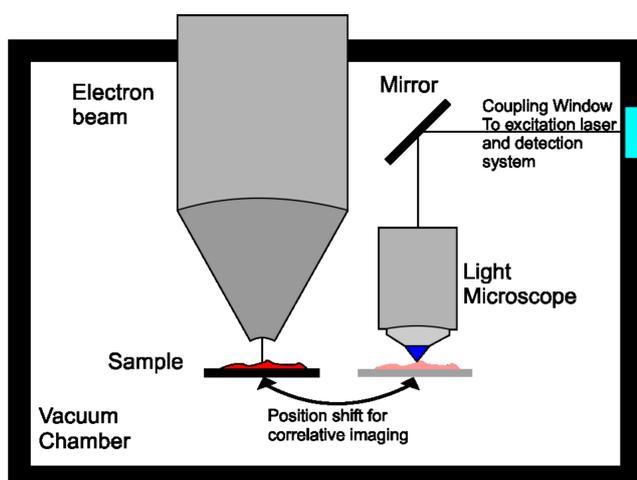


FIG. 2. **Correlative optical electron microscopy.** Schematic of a SEM vacuum chamber with in coupling optics for correlative light microscopy. Reproduced from Ref<sup>3</sup>.

(CLEM) and *integrated* correlative light and electron microscopy (iCLEM)<sup>3</sup>.

This section will begin with a detailed discussion of the methods and technical challenges of integrating these imaging techniques before highlighting examples of insight into relevant systems revealed by these methods.

### B. Electron Microscopy Techniques

Bridging lengthscales. Characterisation of nanoparticles can be done at the atomic scale, but shape/size etc is done at a scale that involves an ensemble measurement. Correlative techniques provide a means to bridge these scales<sup>10</sup>. X-ray microscopy and X-ray absorption spectroscopy provide rich electronic and local geometric information<sup>11</sup>. Other techniques that we will discuss include X-ray fluorescence (XRF) spectroscopy.

### C. Electron Microscopy and Electrochemical Methods

Understanding the structure-reactivity relationship at electrochemical interfaces is central to understanding nearly all electrochemical processes. Here one method is imaging and one is electrochemical. These methods include electrochemical multimicroscopy, where electrochemical imaging is spatially correlated with complementary imaging or microscopic techniques integrated with scanning electrochemical cell microscopy for example<sup>11</sup>. Electrochemical Correlative Microscopy intersects with *operando* electrochemical characterization (performed under working conditions)<sup>12</sup>.

### D. Electron Microscopy and Raman spectroscopy

Raman spectroscopy has been combined with (environmental) TEM where the sample is placed in an at-

mosphere rather than in a vacuum. This opens the possibility to combine the chemical characterization of Raman spectroscopy with the spatial resolution of TEM for soft matter and even wet samples<sup>13</sup>. The Raman technique is also suitable for combination with SEM. SEM is fast, after which Raman analysis may be performed on structures and regions of interest<sup>3</sup>.

### E. Correlative Microscopy for Particulate Systems

Key examples of enhanced characterisation made possible through the use of correlative microscopy. Confocal-AFM methods will be discussed. One example is the use of multiple dyes, that enable not only the particle coordinates to be extracted but also the *forces* between particles (Fig. 3)<sup>14</sup>.

### III. GENERALIZED CORRELATIVE MICROSCOPY

In its strictest sense, correlative microscopy concerns imaging the *same* region of a system with multiple techniques. In this section, we will argue for a broader interpretation, of using multiple techniques in a *complementary* manner. In principle, some might not even be imaging techniques (see Sec. II C above). This broader approach constitutes *Generalized* Correlative Microscopy (GCM).

To give one example, in the context of film formation in water-based (latex) coatings, the author applied environmental SEM to characterize the surface of the drying film with the sample under a water vapor atmosphere<sup>15</sup>. This was combined with 3d confocal microscopy to probe the interior of the film<sup>8</sup> and AFM to probe the mechanical properties of the surface of the dried film<sup>16</sup>. These techniques were applied to different samples of the same system under different conditions the environmental SEM while the film was still wet, the confocal microscopy to the 3d interior of the film and the AFM to the surface of the dried film. All provided *complementary* information which was further combined with macroscopic techniques such as reflectometry.

A second quite popular method of GCM is to use electron microscopy to characterize particles in detail and then lower resolution optical methods (here confocal microscopy) to study their assembly (Fig. 4)<sup>17</sup>.

### IV. EMERGING TECHNIQUES IN THE FIELD.

Here we will discuss new techniques which show future potential. For example, Ultrafast Electron Microscopy (UEM). This provides access to ultrafast structural dynamics with high local selectivity, enabling the exploration of the dynamic nature of chemical bonding in non-equilibrium states<sup>18</sup>.

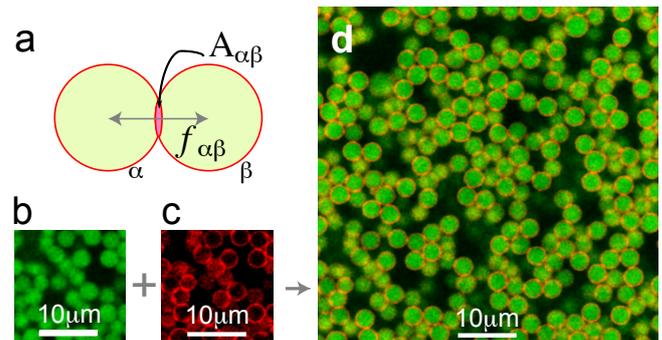


FIG. 3. **Imaging of contacts and forces between droplets in a colloidal gel.** (a) Distribution of solvatochromic dye in two emulsion droplets  $\alpha$  and  $\beta$  at contact. The region shaded in pink  $A_{\alpha,\beta}$  is related to the force between droplets  $f_{\alpha,\beta}$ . (b,c) Two channels showing droplets (green) and contacts (red). (d) Combined two-channel image of gel<sup>14</sup>.

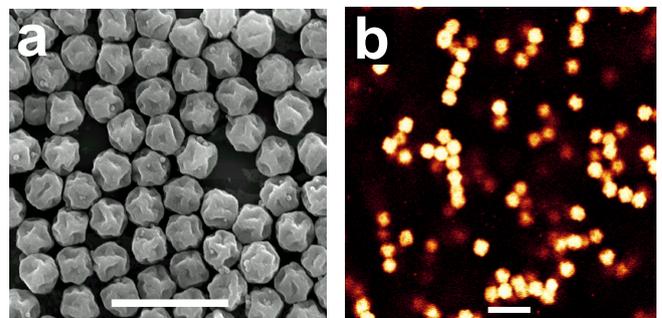


FIG. 4. **Generalised correlative microscopy.** Polyhedral colloidal “rocks” characterised with SEM (a) showing higher resolution detail of the particles. (b) Confocal microscopy of a 3d system from which the coordinates of the “rocks” can be extracted. Scale bars in (a) and (b) are 10  $\mu\text{m}$ <sup>17</sup>.

### APPENDIX. COMMERCIAL SYSTEMS

Here we will provide some details of commercial systems suitable for correlative microscopy. Possible examples include products from Delmic<sup>19</sup>, FEI and Zeiss<sup>20</sup>.

### ACKNOWLEDGEMENTS

The author would like to thank Prof. Wilson Poon for the initial development of this project.

## REFERENCES

- <sup>1</sup>R. E. Webster, M. Osborn, and K. Weber, “Visualization of the same ptk2cytoskeletons by both immunofluorescence and low power electron microscopy,” *Exp. Cell. Res.* **117**, 47–61 (1987).
- <sup>2</sup>L. Limozin and K. Sengupta, “Quantitative reflection interference contrast microscopy (ricm) in soft matter and cell adhesion,” *Chem. Phys. Chem.* **10**, 2752–2768 (2002).
- <sup>3</sup>F. J. Timmermans and C. Otto, “Contributed review: Review of integrated correlative light and electron microscopy,” *Rev. Sci. Instr.* **86**, 011501 (2015).
- <sup>4</sup>S. W. Hell, “Nanoscopy with focused light,” Nobel Lecture (2014).
- <sup>5</sup>S. W. Hell, “Far-field optical nanoscopy,” *Science* **353**, 1163–1168 (2007).
- <sup>6</sup>D. Lyumkis, “Challenges and opportunities in cryo-em single-particle analysis,” *J. Biol. Chem.* **294**, 5181–5197 (2019).
- <sup>7</sup>B. de Nijs, S. Duss, F. Smalenburg, J. D. Meeldijk, D. J. Groenendijk, L. Filion, A. Imhof, A. van Blaaderen, and D. M., “Entropy-driven formation of large icosahedral colloidal clusters by spherical confinement,” *Nature Materials* **14**, 56–60 (2015).
- <sup>8</sup>C. P. Royall and A. M. Donald, “Structure of silica in matte water-based lacquer,” *Phys. Rev. E* **66**, 021406 (2002).
- <sup>9</sup>A. Kawafi, L. Kürten, L. Ortlieb, Y. Yang, A. Mauleon Amieva, J. E. Hallett, and C. P. Royall, “Colloidoscope: Detecting dense colloids in 3d with deep learning,” *Soft Matter* **21**, 6338–6352 (2025).
- <sup>10</sup>S. Neumann and D. Rafaja, “Correlative multi-scale characterization of nanoparticles using transmission electron microscopy,” *Powders* **3**, 531–549 (2024).
- <sup>11</sup>S. Wenzel, R. Garcia-Carrillo, and H. Ren, “Electrochemical correlative microscopy: Discovering insights into structure-reactivity relationships for electrochemical energy conversion and storage,” *Curr. Op. Electrochem.* **50**, 101666 (2025).
- <sup>12</sup>C. Santana Santos, B. Nsolebna Jaato, I. Sanjuán, W. Schuhmann, and C. Corina Andronesu, “Operando scanning electrochemical probe microscopy during electrocatalysis,” *Chem. Rev.* **123**, 4972–5019 (2023).
- <sup>13</sup>M. Picher, S. Mazzucco, S. Blanekshnip, and R. Sharma, “Vibrational and optical spectroscopies integrated with environmental transmission electron microscopy,” *Ultramicroscopy* **150**, 10–15 (2015).
- <sup>14</sup>J. Dong, F. Turci, R. L. Jack, M. A. Faers, and C. P. Royall, “Direct imaging of contacts and forces in colloidal gels,” *J. Chem. Phys.* **156**, 214907 (2022).
- <sup>15</sup>C. P. Royall and A. M. Donald, “Optimisation of the environmental scanning electron microscope for observation of drying of matte water-based lacquers,” *Scanning* **24**, 305–313 (2002).
- <sup>16</sup>C. P. Royall and A. M. Donald, “Surface properties and structural collapse of silica in matte water based lacquers,” *Langmuir* **18**, 9519–9526 (2002).
- <sup>17</sup>R. Rice, R. Roth, and C. P. Royall, “Polyhedral colloidal rocks: low-dimensional networks,” *Soft Matter* **8**, 1163–1167 (2012).
- <sup>18</sup>Y.-J. Kim, W.-W. Park, H. W. Nho, and O.-H. Kwon, “High-resolution correlative imaging in ultrafast electron microscopy,” *Advances in Physics: X* **9**, 1–37 (2024).
- <sup>19</sup>Delmic: <https://www.delmic.com/en/techniques/correlative-light-electron-microscopy>.
- <sup>20</sup>Zeiss: <https://www.zeiss.com/microscopy/en/products/software/zeiss-nanoscale-and-correlative-analysis.html>.