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Real-time Optical Imaging of Electrocatalytic Nanoparticles and their Activity

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Abstract

Using a combination of dark-field microscopy and fluorescence electrochemical microscopy, we follow electrochemical reactions at catalytically active Au@Pt core-shell nanoparticles. Their catalytic activity for oxygen reduction reaction and the associated local decrease of pH at particle surfaces is exposed by a pH-sensitive fluorescence indicator. Simultaneously, the localized surface plasmon resonance was utilized to localize active catalyst particles under operation, and identical location SEM measurement revealed their individual sizes and shapes. We observed how the growth and collapse of the proton depletion zone depend on the size of the active nanoparticle structure, highlighting the fact that electrocatalytic activity is largely dictated by size and nanoscale arrangement of the catalytic nanoparticles. Our approach is universally applicable to reactions involving pH changes and can provide high-throughput screening of catalytic activity with sub-micrometer resolution and will provide systematic single-particle-based insight into the structure-reactivity relationship of catalytic surfaces.

The performance of catalysts relevant for many heterogeneous reactions, for example, electrochemical water splitting, largely depends on their micro- and nanostructure, which affects catalytic activity, mass transport rates, and sorption of reactants and products.^{1–3} The rational design of efficient catalysts can be achieved by specifically tailoring the surface structure.^{4,5} This, however, requires prior knowledge of the effects of nanoscale structure on catalytic activity. To deduce these structure-activity relationships, suitable analytical methods capable of resolving local activity differences on nanoscale catalysts are indispensable.

While many different descriptors can be used to evaluate catalytic activity, our approach exploits the fact that many

catalytic reactions release or consume protons. This includes, among others, the reduction of carbon dioxide (CO₂RR)⁴, nitrogen (N₂RR)⁶, or oxygen (ORR)⁷, the evolution of hydrogen (HER)^{8–10} or oxygen (OER)¹¹, and the conversion of biomass to value-added compounds.^{12,13} Accordingly, nanoparticles catalyzing one of these reactions can be identified by the local change of the electrolyte pH in their direct vicinity. Here, we demonstrate a time-resolved method of characterizing the local catalytic activity at noble metal nanoparticles (NPs) by exploiting local changes of the electrolyte pH due to the proceeding catalytic reaction, revealing a size-dependent consumption of reactants.

Techniques able to resolve the structure of heterogeneous catalysts at the

nanoscale and simultaneously assess activity under reaction conditions are rare. On the one hand, the applicability of liquid cell electron microscopy to investigate electrocatalytic reactions is limited by the interactions between the electron beam, the liquid, and the catalyst under study.^{14,15} Purely electrochemical approaches involving statistical collision events of nanoparticles with ultra-microelectrodes, on the other hand, yield information on the electrocatalytic activity of individual nano-entities, unblurred by the polydispersity found in any nanoparticle ensemble.^{11,16–18} However, this method often fails to provide structural information about the specific particle that caused a given electrocatalytic response. In contrast, the immobilization of a single NP on a nanoelectrode makes the *ex situ* structural characterization by electron microscopy possible, and therefore, the assessment of the catalytic activity of the same NP, yet at the cost of statistical explanatory power, given the ultra-low throughput of this approach.^{19–22}

Another approach is to use spatially resolved electrochemical methods, such as scanning electrochemical microscopy, to image the diffusion layer of reactants/products around a catalytically active nanoscale feature.^{23–25} Again, high-resolution imaging of a statistically relevant number of nanoscale features is challenging and time-consuming. Finally, scanning electrochemical cell microscopy followed by identical location electron microscopy can yield correlated structure-activity data related to individual active nanoscale features, again with *ex situ* structural information.²¹ Nevertheless, structural changes during the reaction, as provided by *operando* measurements, are out of the scope of these techniques. For this reason, we present a combination of two optical microscopic techniques capable of

assessing the reactivity of individual NPs with an inherent possibility of high-throughput data extraction to elucidate structure-activity correlations with statistical significance.

Many metals used for electrocatalysis, such as Au, Ag, or Cu, exhibit plasmonic properties. NPs consisting of these metals provide localized surface plasmon resonance (LSPR) within the visible range of the electromagnetic spectrum, which can be probed by dark-field microscopy (DFM) for the localization of NPs, as well as the assessment of shape-related features via the respective LSPR spectra. Furthermore, the low-cost and high-throughput nature of DFM and the operation under ambient conditions make DFM investigations suitable for numerous plasmonic NP investigations. By coupling DFM with electrochemical setups, changes of single NP entities have been tracked and interpreted for the elucidation of NP transformation mechanisms, such as the formation of AgCl or AgSCN from Ag NPs²⁶ or revealing the effect of chloride ions present near Au NPs during H₂O₂ oxidation catalysis.²⁷ Also, adsorbate formation during hydrazine oxidation at Au NPs was tracked alongside the oxide formation for elucidating the mechanism of this reaction.²⁸ Furthermore, atomic layer deposition, strong adsorption processes, or refractive index changes via, e.g., surface oxide formation have been tracked with DFM.^{10,29–33} However, important regions for the evaluation of catalytic activity, such as the diffusion layer, indicating the formation of products or depletion of reactants, lie outside the scope of conventional DFM.

Conversely, high sensitivity for the determination of even small amounts of product is provided by fluorescence or electrochemiluminescence microscopy, even allowing the detection of single product molecules in reactions.^{34–38}

Concerning the detection of pH changes during electrochemical reactions, fluorescent pH indicators, such as fluorescein, quinine, or 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS), have been utilized due to their high quantum yields, chemical stability, and water solubility.^{39–41} The general principle of this detection method, known as fluorescence electrochemical microscopy (FECM), is reported for various macroscopic setups.⁴² Until now, FECM has not been applied to catalytically active NP structures, although it holds great potential in understanding structure-activity relationships. Especially in combination with DFM, the localization of plasmonic structures and thus identical location SEM investigations are facilitated, which is crucial for correlating the catalytic behavior of active structures with morphological features.

In this work, we resolve local catalytic activity on NP structures, which is made possible by the combination of DFM and FECM. The high-throughput nature of the optical microscopy approach will allow a rapid accumulation of large datasets, while probing active areas with nanometer sizes and readily assign activity to the catalyst structure.

Results

Opto-Electrochemical Measurements

We initially investigate the catalytic activity of Au@Pt core-shell NPs (60 nm diameter, see Figure S1) via fluorescent pH probing. These NPs provide both high catalytic activity towards ORR, as well as plasmonic features in the visible range. Indium-tin-oxide (ITO) was used as the electrode material due to its optical transparency. The DFM allows to probe the LSPR of structures with sizes below the diffraction limit very sensitively, as the blocking of the transmitted light provides a dark background (compare Figure 1). This also makes it suitable for probing

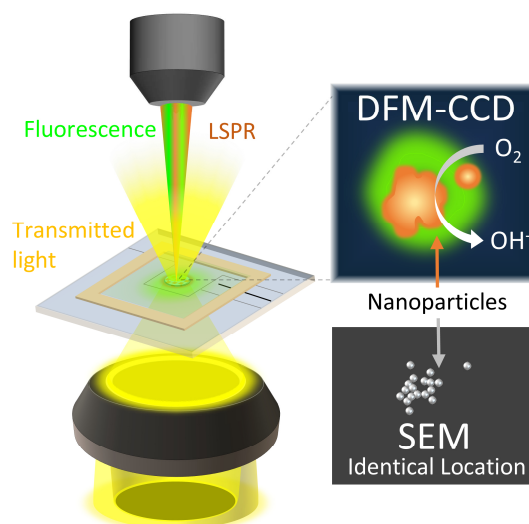


Figure 1: Schematic representation of the dark-field microscope. Partially blocked white light is focussed on the transparent electrochemical cell. Herein transmitted light is blocked, whereas scattered light (orange) and fluorescence (green) can be detected. Fluorescent regions during electrochemical pulses indicate electrocatalytic activity, whereas the LSPR of the NPs facilitates localization and thus identical location SEM studies.

fluorescence, either by imaging using a CCD detector or by hyperspectral analysis using a hyperspectral imaging (HSI) detector. Our measurement approach is illustrated in Figure 1 for imaging catalytic processes at plasmonic NP catalysts immobilized on a non-catalytic ITO-electrode. Local electrocatalytic activity due to the presence of catalytically active NPs will cause local pH changes, which are visualized using the fluorescence response of the pH-sensitive fluorescein dye.

To evaluate the activity difference of Au@Pt NPs compared to untreated ITO, a CCD detector was used to record videos at a selected part of the electrode during reductive linear sweep voltammetry (LSV) measurements before and after Au@Pt NP modification. With increasing ORR turnover during the reductive sweep, the pH near the electrode surface increases due to the consumption of protons, leading to the deprotonation of fluorescein, which results in strong

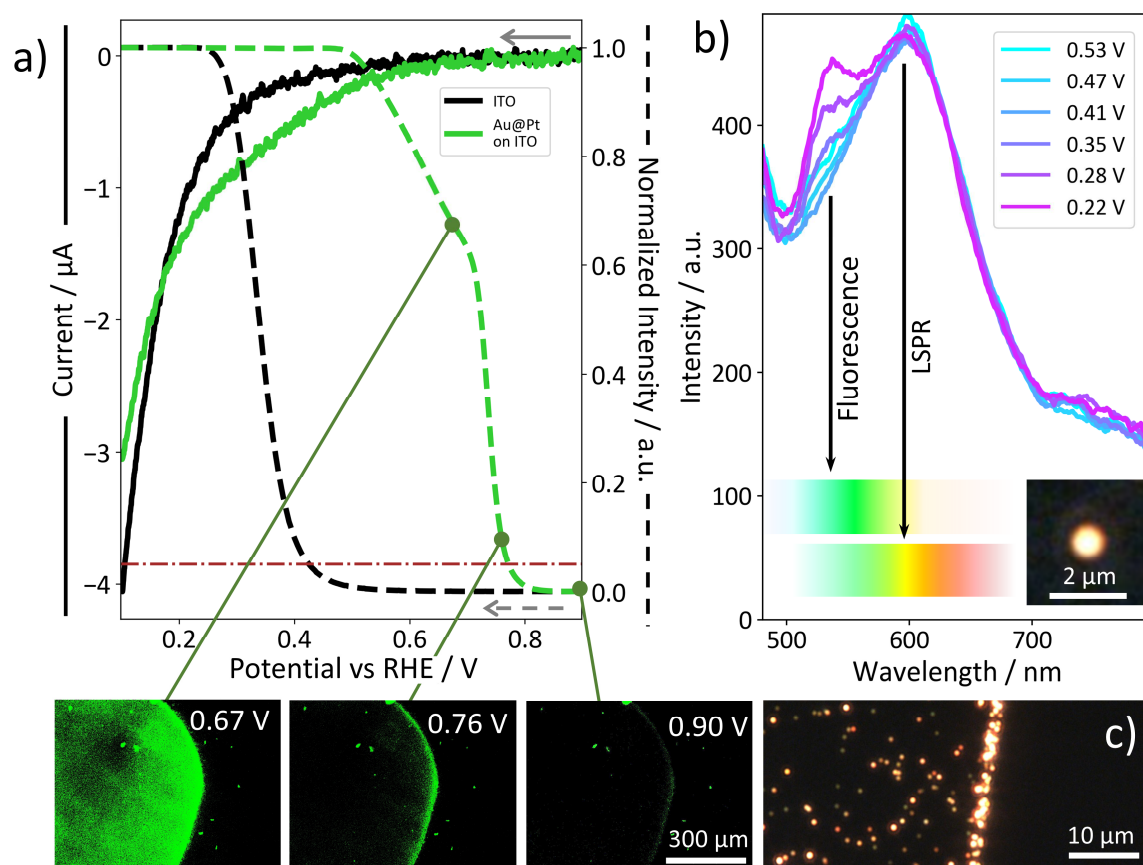


Figure 2: a) Opto-electrochemical linear sweep voltammograms (scan rate 5 mV/s) of Au@Pt NP modified (green) and blank ITO (black) surfaces, where the solid curves represent the current and the dashed curves represent the averaged G-channel intensity of the respective CCD video frame (500 ms exposure time). The scan direction is indicated via grey arrows. The used electrolyte was adjusted to pH 5.1 and contained 40 μ M fluorescein, and 125 mM K_2SO_4 . The images below the graph show CCD images of the monitored region at different potentials. b) Spectral responses of a single Au@Pt NP, which were recorded with a hyperspectral camera. The inset shows the CCD image of the same NP. c) "Coffee Ring" of dropcast Au@Pt NPs as example of highly populated NP regions.

fluorescence signals at catalytically active NPs.^{39,40} We extracted the averaged and normalized fluorescence intensities from the recorded videos and plotted the fluorescence traces in addition to the simultaneously obtained current response (see Figure 2 a)). Here, the normalized fluorescence intensity is displayed as dashed lines, the current response as solid lines, the fluorescence onset (herein defined as the potential at which 5 % of the maximum fluorescence intensity was reached) is indicated by the brown line. Black indicates unmodified ITO, and the green curves correspond to the NP-modified ITO. The fluorescence onset of the NP-modified sample can be observed at significantly less cathodic

potential (0.77 V vs RHE) than for unmodified ITO (0.40 V vs RHE), confirming the higher catalytic activity of the NPs compared to the substrate. Selected frames of the video in Figure 2 a) show that regions of high NP population, such as the "coffee-ring" of a region where NP were dropcast. (compare Figure 2 c)) exhibit fluorescence significantly earlier (0.76 V vs RHE) than the rest of the modified region (0.67 V vs RHE). At more reductive potentials, the diffusion of the fluorescent layer is responsible for the intensity increase and eventual detector saturation, which can be seen in the corresponding video (see video S7-2).

Hyperspectral Investigations

The possibility of probing pH changes and the NP LSPR simultaneously is illustrated in Figure 2 b), where a spectral time series was recorded during a reductive LSV from 0.9 V to 0.2 V vs RHE using the HSI detector at a Au@Pt NP surrounded by the fluorescent electrolyte. With decreasing potential, the time series clearly shows an increasing fluorescence signal at a wavelength of around 520 nm. The large signal at around 600 nm corresponds to the LSPR spectrum of the Au@Pt particle, which remains unchanged by potential. Notably, the LSPR portion of the spectrum contains structural information about the observed NPs themselves since the LSPR frequency depends on particle size and shape. This suggests that within our measurement conditions, the NP does not undergo significant morphological or compositional changes. As the increase of the fluorescence band occurs at < 0.4 V vs RHE, which is the same potential at which the ITO substrate shows pH changes (see Figure S5 in the SI), we attribute the emerging fluorescence mainly to reactions at the underlying substrate and not to reactions at the NP itself.

Activity monitoring of small structures

After successfully monitoring the activity of a large area modified with the Au-based NPs during electrocatalysis (see Figure 2) we explored the catalytic activity of smaller structures. For this, Au@Pt NPs were dropcast on an ITO electrode. Figure 3 A1 and B1 shows DFM CCD images of two differently sized, representative NP clusters (named A and B), which were further studied using local fluorescence pH indication.

To observe the electrocatalytic reaction by the local pH shift, we used short cathodic potential pulses to trigger the

reaction. This ensures a low background fluorescence intensity and avoids local depletion of protons. Instead, the initial conditions are restored a few seconds after the end of each potential pulse. Figure 3 A3 and B3 show the increase of fluorescence intensities (dark yellow color) of two active NP structures during the 300 ms long potential pulses to 0.0 V vs RHE compared to the background (teal color). Herein, the background-corrected intensity (purple color) is the difference between the fluorescence intensities at an active Au@Pt region and fluorescence intensity at the ITO background. The background-corrected intensity of cluster A shows a slow increase of fluorescence intensity during the pulse, whereas NP cluster B exhibits a steady state behavior. We reason this behavior of smaller structures is due to a quicker emergence of a steady-state diffusion layer compared to larger structures.

The DFM images allow us to localize active NP structures and recognize them in identical-location SEM experiments to further elucidate structural features. As can be seen in Figure 3 A2, the large structure A consists of several NP clusters over an extension of a few micrometers. The smaller structure B, marked with the yellow arrows in Figure 3 B2, has an average extension of roughly 500 nm.

To show the dynamics of the proton depletion layer due to electrocatalytic activity, the recorded videos (see S7-3 and S7-4) were processed by difference-image analysis. The RGB arrays of each pixel were converted into grayscale, and difference images were obtained by subtracting from each frame a preceding frame (by 100 ms). Note that the difference images show an approximation of the derivative of the fluorescence intensity with respect to time and thus are a measure of growing or shrinking proton depletion layers due

to electrocatalytic activity. For visualization, at the positions of pixels with positive differences (growth of fluorescent layer), the difference values were stored in the R-channel of the output image. At the position of pixels with negative intensity differences (shrinking of the fluorescent layer), the difference values were stored in the B-channel of the output image. Additionally, the grayscale-transformed initial frame of the video before applying any potential is stored in the G-channel of the output image to keep the information about the shape and size of

the NP aggregates, as well as the positions of individual nanoparticles in the output image.

Figure 3 A4 and B4 show the positive difference (fluorescence intensity increase) at the start of a potential pulse, corresponding to the growth of the fluorescent layer when protons are depleted due to ORR. Figure 3 A5 and B5 show the negative difference (fluorescence intensity decrease) at the end of the potential pulse, corresponding to the shrinking of the fluorescent layer when the catalytic

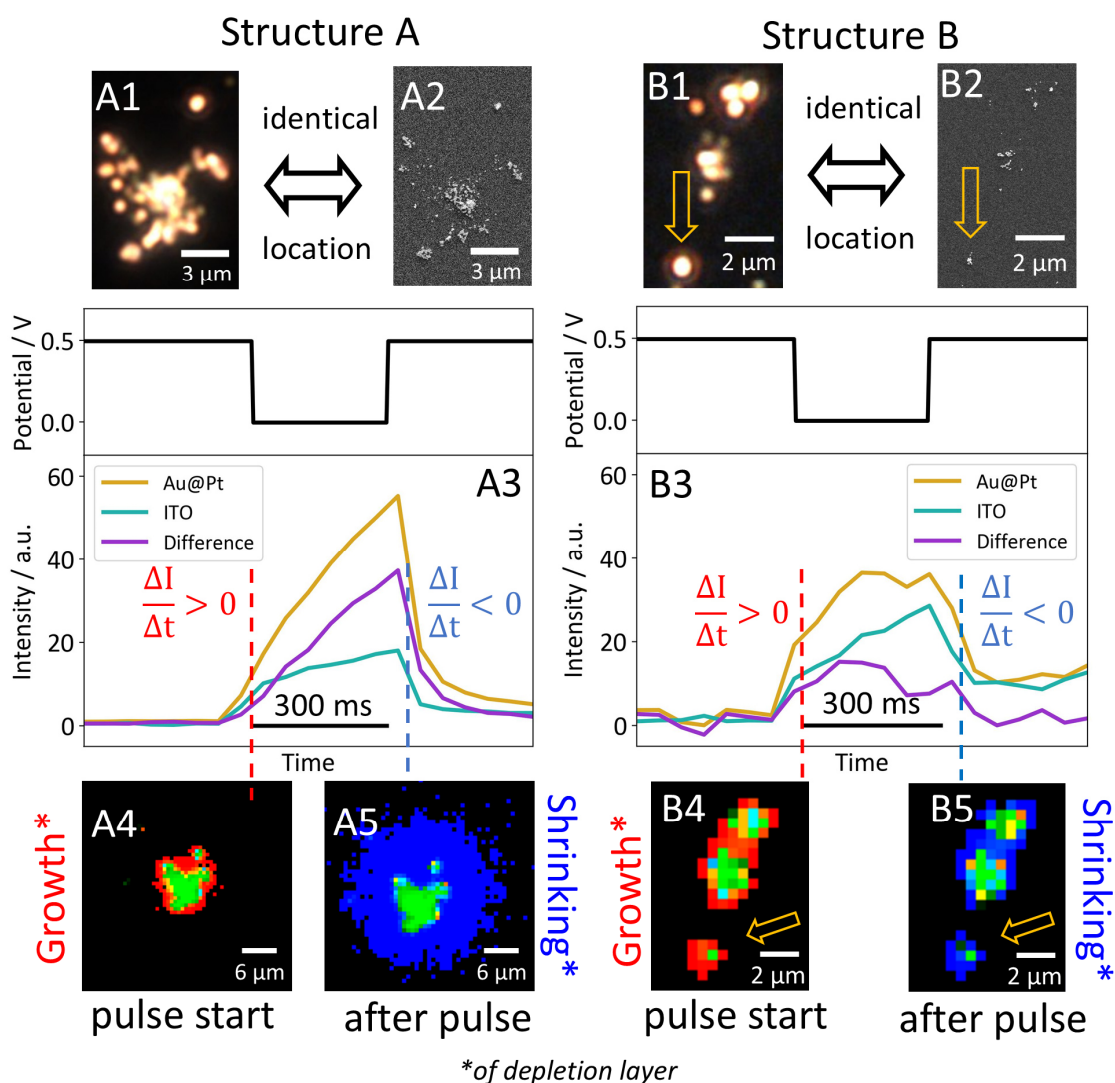


Figure 3: DFM, SEM and FECM analysis of two differently sized active NP structures A and B: 1) DFM Images recorded in electrolyte without fluorescein (50 ms exposure time, 100x magnification) and 2) the SEM image of the same location. 3) Intensity values extracted via regions of interest functionality around the structures (dark yellow) including background intensity (teal) and background-corrected intensities (purple). 4) Difference image before the potential pulse at 0.0 V vs RHE and 5) difference image after the 300 ms pulse. The difference images show positive differences as red pixels and negative differences as blue pixels, which represents the growing and shrinking of the fluorescent region. Yellow arrows mark the position of the structure B.

reaction has ceased. These effects are compared at a larger active structure of individual particles combined to an aggregate of several μm in size (A1-A5) and a smaller, nanoscale structure (B1-B5). Interestingly, for agglomerate A, the negative difference at the end of the pulse (fluorescent layer shrinking) shown in blue (Figure 3 A5) exhibits a larger spatial extension than the positive difference shown in Figure 3 A4 (fluorescent layer growth). This might be explained by the rapid diffusion of protons supported by the buffering of the electrolyte after the catalytic reaction (ORR), responsible for the pH increase, is stopped. In contrast to that, building up the fluorescent layer is a slower process, leading to less pronounced differences in Figure 3 A4 compared to the vanishing of the fluorescent layer. This trend was found for structures above 3 μm in size, where smaller structures showed no different behavior between growing and shrinking of the fluorescent region, as shown in Figure 3 B4 and B5. This can be explained by the time needed to reach a steady state diffusional flux, which is shorter for smaller active structures.

Discussion

We describe the high potential of a universal methodology based on DFM and FECM to resolve local catalytic activity at the nanoscale on heterogeneous catalyst surfaces. The method used for the study combines the high sensitivity of DFM towards the localization of plasmonically active NP structures with the possibility of assessing catalytic activity by FECM. As one example, we analyzed the activity towards the ORR at catalytically active Au@Pt NPs using a pH-sensitive fluorescent dye to visualize proton depletion as a measure of electrocatalytic turnover. We observe that the depletion layer evolves at different rates when comparing active

nanoscale catalysts to larger aggregates. Having developed and showcased this powerful approach for the ORR at Pt nanoparticles, we note that the methodology can be readily exploited to investigate structure-activity relations at nanocatalysts for a broad range of proton-consuming reactions, such as CO_2RR , HER, and N_2RR . Jointly with the fact that local LSPR spectra recorded by DFM contain structural information about the size and shape of individual NPs, this approach holds great potential for high-throughput studies linking the structure of individual nanocatalysts to their catalytic activity and thus elucidating the link between these properties.

Methods

Chemicals

Fluorescein sodium salt (91.3 %) was purchased from Sigma-Aldrich, potassium sulphate (99.3 %) was purchased from VWR Chemicals, potassium hydroxide (86.7 %) and sulphuric acid (95.6 %) were purchased from Chempur. ITO-covered glass slides were purchased from Sigma-Aldrich (4 cm^2 area, 8-12 Ω/sq surface resistivity). Au@Pt NPs were synthesized by our group; the synthesis procedures can be found in S1.⁴³

All solutions were prepared with ultrapure water, deionized by a Thermo Scientific Barnstead GenPure xCAD Plus (conductivity < 0.055 $\mu\text{S}\cdot\text{cm}^{-2}$ at 23 °C). A solution of 125 mM K_2SO_4 and 40 μM Fluorescein was used for the FECM experiments. For different dilutions, a 125 mM K_2SO_4 solution was used as a diluting agent to maintain the same ionic strength in all solutions. The pH values of the test solutions were adjusted with 0.1 M solutions of KOH or H_2SO_4 by using a SevenCompact pH meter S220 equipped with a LE410 pH-electrode (Mettler Toledo).

Dark-Field Microscope

The DFM (Cytoviva Inc.) was based on an Olympus BX43 Microscope (Olympus Deutschland GmbH). We use a 100-fold magnification oil immersion iris objective (UPlanFL N 100x/0.6-1.30 Oil Iris) or a 10-fold magnification air objective (10x Plan Achromat) and a Retiga R1 OEM Camera as optical detector. The immobilized NPs were observed with 10-fold and 100-fold magnification, and images were recorded using Ocular™ Scientific Acquisition Software. Furthermore, hyperspectral imaging was performed using a Specim ImSpector V10E Spectrograph in combination with a Cooke Pixelfly Spectral Camera. Switching between the detectors is possible by the use of a beam splitter. A halogen lamp was used as a light source. For the measurements, we brought a custom-made spectro-electrochemical cell into contact with the DFM condenser using immersion oil (Cargille, Type A, refractive index of the sodium D-line of 1.5150 at 23 °C), see Figure 1. For 100-fold magnification, immersion oil was also placed between the coverslip of the cell and the respective objective.

Electrochemical measurements

Electrochemical measurements were performed using a potentiostat (OrigaFlex OGF500, Origalys). The measurements shown in Figure 2 were recorded using a Pt quasi-RE; the measurement in Figure 3 was recorded using an Ag/AgCl 3 M KCl RE. For experiments using a Pt quasi-RE, the converted potentials against RHE might be prone to some extent of uncertainty due to possible potential drift of the Pt quasi-RE.

Electrolyte preparation

We first optimized the electrolyte used for the FECM approach using a Pt wire

as the working electrode (WE) (see Figure S2 and Figure S3 in the SI). Herein, pH values between 4.8 and 5.6 and dye concentrations of approximately 40 μM provided a good combination of sensitivity and signal intensity for probing fluorescence responses induced by pH changes. Before analysis, NPs were dropcast on an ITO electrode as indicated in Figure 1 B.

Scanning Electron Microscope

For the SEM imaging, a JEOL IT800 device equipped with an EDAX Genesis XM2i EDX system using the AZtec analysis software was used. Herein, an acceleration voltage of 15.00 kV was used. The SEM image in Figure 3 A2 was recorded with a magnification of 1.9 k at a working distance of 9.3 mm, and for Figure 3 A3, a magnification of 6.00 k at a working distance of 9.2 mm was used.

Cell preparation

For the fabrication of the electrochemical cells, ITO slides were processed by electrically separating the electrode regions by mechanical scratching of the surface or masking the ITO with patterned vinyl foil with subsequent HCl etching (37 % HCl for 10 minutes). ITO always served as the counter electrode (CE), whereas, depending on the experiment, Pt-wire or ITO was used as the working electrode (WE) material. As the reference electrode (RE), either a Pt quasi-RE (QRE) was used, as indicated in Figure 1 B, or the cell was connected to an external tubing including an Ag/AgCl 3 M KCl electrode.

The NPs were immobilized on the ITO surface by dropcasting (0.3 μL of undiluted suspension) on the WE region of the electrochemical cell (Figure 1 B). For the targeted generation of small NP agglomerates on the electrode, tilting

the ITO slide during the drying process of the NP suspension ensured an inhomogeneous distribution of the NP entities over the modified region during the drying process.

References

1. Ma, Y. & Li, D. Recent advances in characterization of electrocatalytic nanoparticles at single-particle level. *Curr. Opin. Electrochem.* **49**, 101623; 10.1016/j.coelec.2024.101623 (2025).
2. Yang, L. *et al.* Single Nanoparticle Collision Electrocatalysis Driven by Ultrafast High-Temperature Precision Synthesis. *Nano Lett.* **25**, 8320–8326; 10.1021/acs.nanolett.5c01519 (2025).
3. Anderson, T. J. & Zhang, B. Single-Nanoparticle Electrochemistry through Immobilization and Collision. *Acc. Chem. Res.* **49**, 2625–2631; 10.1021/acs.accounts.6b00334 (2016).
4. Mao, X., Sarkar, M. & He, J. Design of polymer–metal nanocatalyst interfaces for the electrochemical CO₂ reduction reaction. *Journal of Materials Chemistry A* **14**, 11848–11879; 10.1039/D5TA09312K (2026).
5. Jin, H. *et al.* Nanocatalyst Design for Long-Term Operation of Proton/Anion Exchange Membrane Water Electrolysis. *Adv. Energy Mater.* **11**, 2003188; 10.1002/aenm.202003188 (2021).
6. Wen, J. *et al.* Nanomaterials for the electrochemical nitrogen reduction reaction under ambient conditions. *Nanoscale Advances* **3**, 5525–5541; 10.1039/D1NA00426C (2021).
7. Li, S. *et al.* Selective oxygen reduction reaction: mechanism understanding, catalyst design and practical application. *Chemical science* **15**, 11188–11228; 10.1039/D4SC02853H (2024).
8. Zou, X. & Zhang, Y. Noble metal-free hydrogen evolution catalysts for water splitting. *Chem. Soc. Rev.* **44**, 5148–5180; 10.1039/c4cs00448e (2015).
9. Zhu, J., Hu, L., Zhao, P., Lee, L. Y. S. & Wong, K.-Y. Recent Advances in Electrocatalytic Hydrogen Evolution Using Nanoparticles. *Chem. Rev.* **120**, 851–918; 10.1021/acs.chemrev.9b00248 (2020).
10. Xu, S. *et al.* Real-Time Visualization of the Single-Nanoparticle Electrocatalytic Hydrogen Generation Process and Activity under Dark Field Microscopy. *Anal. Chem.* **92**, 9016–9023; 10.1021/acs.analchem.0c01129 (2020).
11. Liu, Z. *et al.* Facet-Dependent Intrinsic Activity of Single Co₃O₄ Nanoparticles for Oxygen Evolution Reaction. *Adv. Funct. Mater.* **33**; 10.1002/adfm.202210945 (2023).
12. Bondue, C. J., Spallek, M., Sobota, L. & Tschulik, K. Electrochemical Aldehyde Oxidation at Gold Electrodes: gem-Diol, non-Hydrated Aldehyde, and Diolate as Electroactive Species. *ChemSusChem* **16**, e202300685; 10.1002/cssc.202300685 (2023).
13. Werpy, T. & Petersen, G. Top Value Added Chemicals from Biomass: Volume I -- Results of Screening for Potential Candidates from Sugars and Synthesis Gas, 2004.
14. Fritsch, B. *et al.* The Influence of Ionizing Radiation on Quantification for In Situ and Operando Liquid-Phase Electron Microscopy. *Adv. Mater.* **37**, e2415728; 10.1002/adma.202415728 (2025).
15. Shen, T.-H., Girod, R., Vavra, J. & Tileli, V. Considerations of Liquid-Phase Transmission Electron Microscopy Applied to Heterogeneous Electrocatalysis. *J. Electrochem. Soc.*

- 170**, 56502; 10.1149/1945-7111/acced4 (2023).
16. Azimzadeh Sani, M. & Tschulik, K. Unveiling colloidal nanoparticle properties and interactions at a single entity level. *Curr. Opin. Electrochem.* **37**, 101195; 10.1016/j.coelec.2022.101195 (2023).
17. Vieira, M. M. *et al.* Nanoimpact Electrochemistry to Quantify the Transformation and Electrocatalytic Activity of Ni(OH)₂ Nanoparticles: Toward the Size-Activity Relationship at High Throughput. *J. Phys. Chem. Lett.* **13**, 5468–5473; 10.1021/acs.jpcclett.2c01408 (2022).
18. Zhong, R. *et al.* From Ensemble Electrochemistry to Nano-Impact Electrochemistry: Altered Reaction Selectivity. *Angew. Chem. Int. Ed.* **61**, e202207270; 10.1002/anie.202207270 (2022).
19. Brasiliense, V. *et al.* Opto-electrochemical In Situ Monitoring of the Cathodic Formation of Single Cobalt Nanoparticles. *Angew. Chem. Int. Ed.* **56**, 10598–10601; 10.1002/anie.201704394 (2017).
20. Huang, K., Clausmeyer, J., Luo, L., Jarvis, K. & Crooks, R. M. Shape-controlled electrodeposition of single Pt nanocrystals onto carbon nanoelectrodes. *Faraday Discuss.* **210**, 267–280; 10.1039/c8fd00018b (2018).
21. Quast, T. *et al.* Single Particle Nanoelectrochemistry Reveals the Catalytic Oxygen Evolution Reaction Activity of Co₃O₄ Nanocubes. *Angew. Chem. Int. Ed.* **60**, 23444–23450; 10.1002/anie.202109201 (2021).
22. Yu, Y. *et al.* Electrochemistry and Electrocatalysis at Single Gold Nanoparticles Attached to Carbon Nanoelectrodes. *ChemElectroChem* **2**, 58–63; 10.1002/celec.201402312 (2015).
23. Huang, K., Anne, A., Bahri, M. A. & Demaille, C. Probing individual redox PEGylated gold nanoparticles by electrochemical--atomic force microscopy. *ACS Nano* **7**, 4151–4163; 10.1021/nn400527u (2013).
24. O'Connell, M. A. & Wain, A. J. Mapping electroactivity at individual catalytic nanostructures using high-resolution scanning electrochemical-scanning ion conductance microscopy. *Anal. Chem.* **86**, 12100–12107; 10.1021/ac502946q (2014).
25. Sun, T., Yu, Y., Zacher, B. J. & Mirkin, M. V. Scanning electrochemical microscopy of individual catalytic nanoparticles. *Angew. Chem. Int. Ed.* **53**, 14120–14123; 10.1002/anie.201408408 (2014).
26. Wonner, K., Evers, M. V. & Tschulik, K. Simultaneous Opto- and Spectro-Electrochemistry: Reactions of Individual Nanoparticles Uncovered by Dark-Field Microscopy. *J. Am. Chem. Soc.* **140**, 12658–12661; 10.1021/jacs.8b02367 (2018).
27. Jing, C. *et al.* New insights into electrocatalysis based on plasmon resonance for the real-time monitoring of catalytic events on single gold nanorods. *Anal. Chem.* **86**, 5513–5518; 10.1021/ac500785u (2014).
28. Ma, Y., Highsmith, A. L., Hill, C. M. & Pan, S. Dark-Field Scattering Spectroelectrochemistry Analysis of Hydrazine Oxidation at Au Nanoparticle-Modified Transparent Electrodes. *J. Phys. Chem. C* **122**, 18603–18614; 10.1021/acs.jpcc.8b05112 (2018).
29. Zheng, X. *et al.* Catalytic Gold Nanoparticles for Nanoplasmonic Detection of DNA Hybridization. *Angew. Chem.* **123**, 12200–12204; 10.1002/ange.201105121 (2011).

30. Liu, T., Li, M., Wang, Y., Fang, Y. & Wang, W. Electrochemical impedance spectroscopy of single Au nanorods. *Chemical science* **9**, 4424–4429; 10.1039/c8sc00983j (2018).
31. Jeon, H. B., Tsalu, P. V. & Ha, J. W. Shape Effect on the Refractive Index Sensitivity at Localized Surface Plasmon Resonance Inflection Points of Single Gold Nanocubes with Vertices. *Sci. Rep.* **9**, 13635; 10.1038/s41598-019-50032-3 (2019).
32. Dondapati, S. K. *et al.* Voltage-induced adsorbate damping of single gold nanorod plasmons in aqueous solution. *Nano Lett.* **12**, 1247–1252; 10.1021/nl203673g (2012).
33. Hu, S. *et al.* Observing atomic layer electrodeposition on single nanocrystals surface by dark field spectroscopy. *Nature communications* **11**, 2518; 10.1038/s41467-020-16405-3 (2020).
34. Zhou, C. *et al.* Photoelectrochemical Charge Transfer Imaging at Nanoscale Catalysts. *ACS Energy Lett.* **10**, 3519–3525; 10.1021/acsenenergylett.5c01296 (2025).
35. Lelek, M. *et al.* Single-molecule localization microscopy. *Nat. Rev. Methods Primers* **1**, 39; 10.1038/s43586-021-00038-x (2021).
36. Zhao, M. *et al.* Long-range enhancements of micropollutant adsorption on metal-promoted photocatalysts. *Nat. Catal.* **7**, 912–920; 10.1038/s41929-024-01199-0 (2024).
37. Mao, X., Liu, C., Hesari, M., Zou, N. & Chen, P. Super-resolution imaging of non-fluorescent reactions via competition. *Nat. Chem.* **11**, 687–694; 10.1038/s41557-019-0288-8 (2019).
38. Sambur, J. B. *et al.* Sub-particle reaction and photocurrent mapping to optimize catalyst-modified photoanodes. *Nature* **530**, 77–80; 10.1038/nature16534 (2016).
39. Pande, N. *et al.* Electrochemically Induced pH Change: Time-Resolved Confocal Fluorescence Microscopy Measurements and Comparison with Numerical Model. *J. Phys. Chem. Lett.* **11**, 7042–7048; 10.1021/acs.jpcclett.0c01575 (2020).
40. Rudd, N. C. *et al.* Fluorescence Confocal Laser Scanning Microscopy as a Probe of pH Gradients in Electrode Reactions and Surface Activity. *Anal. Chem.* **77**, 6205–6217; 10.1021/ac050800y (2005).
41. Vitt, J. E. & Engstrom, R. C. Imaging of Oxygen Evolution and Oxide Formation Using Quinine Fluorescence. *Anal. Chem.* **69**, 1070–1076; 10.1021/ac960816b (1997).
42. Yang, M., Batchelor-McAuley, C., Kätelhön, E. & Compton, R. G. Reaction Layer Imaging Using Fluorescence Electrochemical Microscopy. *Anal. Chem.* **89**, 6870–6877; 10.1021/acs.analchem.7b01360 (2017).
43. Cignoni, P. *et al.* Validating Electrochemical Active Surface Area Determination of Nanostructured Electrodes: Surface Oxide Reduction on AuPd Nanoparticles. *J. Electrochem. Soc.* **170**, 116505; 10.1149/1945-7111/ad09f8 (2023).

Author Contributions

Lars Fabian Brämer performed the majority of experiments, analyzed the data and contributed to writing the manuscript.

Kevin Wonner and Paolo Cignoni contributed to developing the optical microscopy method

Dean-Robin Nettler synthesized the analyzed nanoparticles

Gabriel Boitel-Aullen performed identical location SEM measurements

Sumukh Shankar Sharadaprasad contributed to developing the optical microscopy method

Jan Clausmeyer contributed to data analysis and to writing the manuscript

Kristina Tschulik conceptualized and supervised the project and contributed to writing the manuscript

Competing Interests

The authors declare no competing interests.

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Materials & Correspondence

Supplementatry information

Correspondence and request for materials should be addressed to Kristina Tschulik.

Data availability

Supplementary Files

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- [DifferenceVideoFigure3A.avi](#)
- [FBSISubmission.pdf](#)
- [S74Fig3A.mp4](#)
- [IntensityAgglomerateB.csv](#)
- [S73Fig3B.mp4](#)
- [S71ITObankLSV.mp4](#)
- [Figure2bspectra.txt](#)
- [S72AuPtLSV.mp4](#)