Detection of low molecular weight compounds using reflected light microscopy

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While cellular heterogeneity (including metabolic heterogeneity) and single cell analysis attract increasing attention [1], analytical tools which are suitable to monitor the chemical composition of the extracellular space with high spatial and high temporal resolution are still scarce.

In an attempt to address this problem, we have turned to redox hydrogels obtained by crosslinking a redox enzyme and a redox polymer. Thin layers of such redox hydrogels are interesting because their oxidation state is changed by the main substrate of the enzyme they contain and can also be changed by electrochemical means (*i.e.*, with electrodes set to a certain potential). With these in mind, we investigated 1.) if changes of the oxidation state of redox hydrogel thin layers are paralleled by changes of the optical properties of such layers, and 2.) if changes of the optical properties of redox hydrogel thin layers can be observed with reflected light microscopy. We discovered that redox hydrogel thin layers do change their optical properties (*e.g.*, their refractive indices) when their oxidation state is changed. We also discovered that the optical properties of redox hydrogel thin layers can be monitored with reflected light microscopy once such layers are deposited onto transparent substrates (*e.g.*, 170 μm thick cover glass). Building on these two findings, we investigated the optical detection of hydrogen peroxide and glucose with redox hydrogel thin layers containing horseradish peroxidase and glucose oxidase, respectively. By combining these two redox hydrogel thin layers with reflected light microscopy, it was possible to detect hydrogen peroxide in concentrations as low as 12.5 μM, with a spatial resolution of 12 μm × 12 μm, and glucose in concentrations as low as 200 μM, with a spatial resolution of 18 μm × 18 μm [2].

In the next step of our study, we are going to use the developed analytical tool, and its high spatial resolution, at cellular level. Observing the hydrogen peroxide production by cells experiencing oxidative stress and the increased glucose consumption of tumor cells with single cell resolution are envisaged.

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References

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