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Optical pump-probe microscopy for biomedicine and art conservation

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Nonlinear optical microscopy can provide contrast in highly heterogeneous media and a wide range of applications has emerged, primarily in biology, medicine, and materials science. Compared to linear microscopy methods, the localized nature of nonlinear interactions leads to high spatial resolution, optical sectioning, and larger possible imaging depth in scattering media. However, nonlinear contrast (other than fluorescence, harmonic generation or CARS) is generally difficult to measure because it is overwhelmed by the large background of detected illumination light. This background can be suppressed by using femtosecond pulse or pulse train shaping to encode nonlinear interactions in background-free regions of the frequency spectrum. We have developed this shaping technology to study novel intrinsic structural and molecular contrast in biological tissue, generally using less power than a laser pointer. For example we have recently been able to sensitively measure detailed transient absorption dynamics of melanin sub-types in a variety of skin lesions, showing clinically relevant differences of melanin type and distribution between cancerous and benign tissue.[1] Recently we have also applied this technology to paint samples and to historic artwork in order to provide detailed, depth-resolved pigment identification. Initial studies in different inorganic and organic pigments have shown a rich and pigment-specific nonlinear absorption signature.[2] Some pigments, for example lapis lazuli (natural ultramarine), even show marked differences in signal depending on its geographic origin and on age, demonstrating the potential of this technique to determine authenticity, provenance, technology of manufacture, or state of preservation of historic works of art.

[1]Matthews et al., *Sci. Transl. Med.* **3**, 71ra15 (2011).
[2]Samineni et al., *Opt. Lett.* **37**, 1310 (2012).