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provides thousands of point detectors and enables to record in each pixel the in-

of the cell. An EM-CCD camera placed perpendicular to the light sheet pro-

light sheet microscopy to increase the penetration depth, allowing long-term imaging of cells

deep inside of live embryos. Here we show the application of this novel imaging

technology to record the cellular and protein dynamics in developing embryos. The

combination of subcellular spatial resolution, high acquisition speed, and high penetration depth allows study of the spatio-temporal organization and control of cells and proteins that are critical in the development of an organism.

Fluorescence correlation spectroscopy (FCS) is a confocal microscopy-based

method allowing to assess diffusion, transport and interaction properties of

molecules (proteins, nucleic acids, compounds) in vitro and in vivo. Commercial-

ally available instruments enable routine measurements at one or few specific

points inside living cells.

FCS experiments inside living cells or embryos remain a challenge since point

measurements often feature large errors caused by the heterogeneous environ-

ment of the sample. Moreover, biological noise due to cell-to-cell differences of

physical and biological parameters (viscosity, protein expression) induces further variations that are difficult to separate from measurement error. Cur-

rently, these problems are addressed by performing statistical data analysis of

measurements from many cells. To significantly improve the method, FCS

measurements can be conducted simultaneously in many points per cell.

Here we present a recently introduced microscopy setup [1] that allows spa-
tially resolved FCS measurements in 2D optical sections across cells. The setup

is based on a thin diffraction-limited light sheet that illuminates a cross-section of the cell. An EM-CCD camera placed perpendicular to the light sheet pro-\n
vides thousands of point detectors and enables to record in each pixel the in-

coming photons with single photon sensitivity, sub-millisecond time

resolution and close-to-confocal spatial resolution.

We have used this and a confocal setup to perform measurements of the diffusion-

and binding-related mobility of chromatin-forming and -associated GFP-

labeled proteins inside nuclei of living cells in interphase. We could identify and characterize the binding of heterochromatin protein 1 and of histone pro-
teins to chromatin as well as the local dynamics of the chromatin fiber in dif-

different nuclear localisations, supporting the existence of subchromosomal domains with distinct properties.


In Vivo Metabolic Mapping of Stem Cells and Differentiated Progeny in Small Intestine and Colon Crypts by Phasor Fluorescence Lifetime Microscopy

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We have performed label-free Phasor Fluorescence lifetime microscopy (FLIM) to reconstruct the three dimensional metabolic signature of small intestines and colon tissue in vivo. Our method provides a label-free identification and metabolic mapping of stem cells during differentiation. Freshly excised tissues are imaged with two photon microscopy and FLIM within two hours. Lgr5-GFP mice are used to mark the Lgr5+ stem cell population at the base of small intestine (SI) and colon crypts. Using Phasor FLIM analysis of live tissue excited at 880nm and 740nm, we identify and map the concentration of different intrinsic metabolic fluorophores and extracellular matrix elements such as NADH, FAD, and collagen. We observe that different compartiments of the tissue are defined by unique Phasor FLIM signatures. We can distinguish collagen fibers at the base of the crypts, the lamina propria, the vascular network and the epithelium. The FLIM signature at the base of the crypt at 740nm follows exactly the map of stem cells intercalated between adjacent Paneth cells. Paneth cells are characterized by a different FLIM signature with respect to the stem cells thus indicating a difference in the concentration and/or composition of intrinsic fluorophores. The FLIM Z-stack reveals a shift of the metabolic signature of crypt epithelial cells during differentiation. Stem cells at the base of the crypt have the shortest lifetime and the highest NADH/ NAD+ ratio. Movement up the crypt to transit amplifying cells and fully differentiated cells on the mucosal surface corresponds to different FLIM signatures that correspond to decreasing NADH/ NAD+ ratios, as is expected during differentiation.

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