



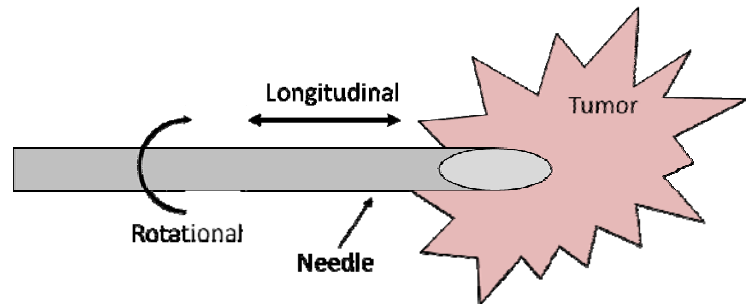
FLUODIAMON is a Collaborative Project funded by the European Commission within the seventh framework programme theme [HEALTH-2007-1.2-2 / Novel optical methodologies for detection, diagnosis and monitoring of disease or disease-related processes]. FLUODIAMON started 2008-06-01, ended 2012-05-31, and includes twelve beneficiaries (PIs with email addresses given in parenthesis):

Beneficiary Number	Beneficiary name	Beneficiary short name	Country
1 (coordinator)	Royal Institute of Technology, Stockholm (Jerker Widengren, jwideng@kth.se)	KTH	Sweden
2	Neodynamics AB (previously VibraTech AB), Stockholm (Hans Wiksell, hans.wiksell@comair.se)	VITECH	Sweden
3	Karolinska Institutet, Stockholm (Gert Auer, gert.auer@ki.se)	KI	Sweden
4	Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V., Institute for Biophysical Chemistry, Göttingen (Stefan W Hell, shell@gwdg.de)	MPiBPC	Germany
5	Heinrich-Heine University, Düsseldorf (Claus AM Seidel, cseidel@hhu.de)	HHU	Germany
6	SensL Inc, Cork (Carl Jackson, cjackson@sensl.com)	SENSL	Ireland
7	Becker&Hickl GmbH, Berlin (Wolfgang Becker, becker@becker-hickl.com)	B&H	Germany
8	University of Luebeck, Lübeck (Jens Habermann, jens.habermann@gmail.com)	LUEBECK	Germany
9	University of Siegen, Siegen (Karl-Heinz Drexhage, drexhage@chemie.uni-siegen.de)	UNISI	Germany
10	University of Turku (Pekka Hänninen, pekka.hanninen@utu.fi)	UTURKU	Finland
11	University of Helsinki (Sampsa Hautaniemi, sampsa.hautaniemi@helsinki.fi)	UH	Finland
12	Academisch Ziekenhuis Leiden (Leiden University Medical Center) (Silvére van der Maarel, S.M.Maarel@lumc.nl)	LUMC	Netherlands

The project is coordinated by KTH, Stockholm, Sweden, represented by Prof. Jerker Widengren (jwideng@kth.se). The public homepage of the project can be found on <http://www.biomolphysics.kth.se/fluodiamon>. The expertise needed for this project is multidisciplinary and comprises clinical cytology, cancer proteomics, molecular biotechnology, fluorophore chemistry, fluorescence microscopy, nanotechnology, optics, solid state detector technology, data processing, and bioinformatics. The level and width of competence required cannot be found on a national level but requires a European initiative.

FIGURES WITH CAPTIONS (AS REFERRED TO IN THE FINAL REPORT):

Figure 1: Fine-needle with added mechanical oscillations facilitating needle penetration, control and manoeuvrability, as well as sample yield.



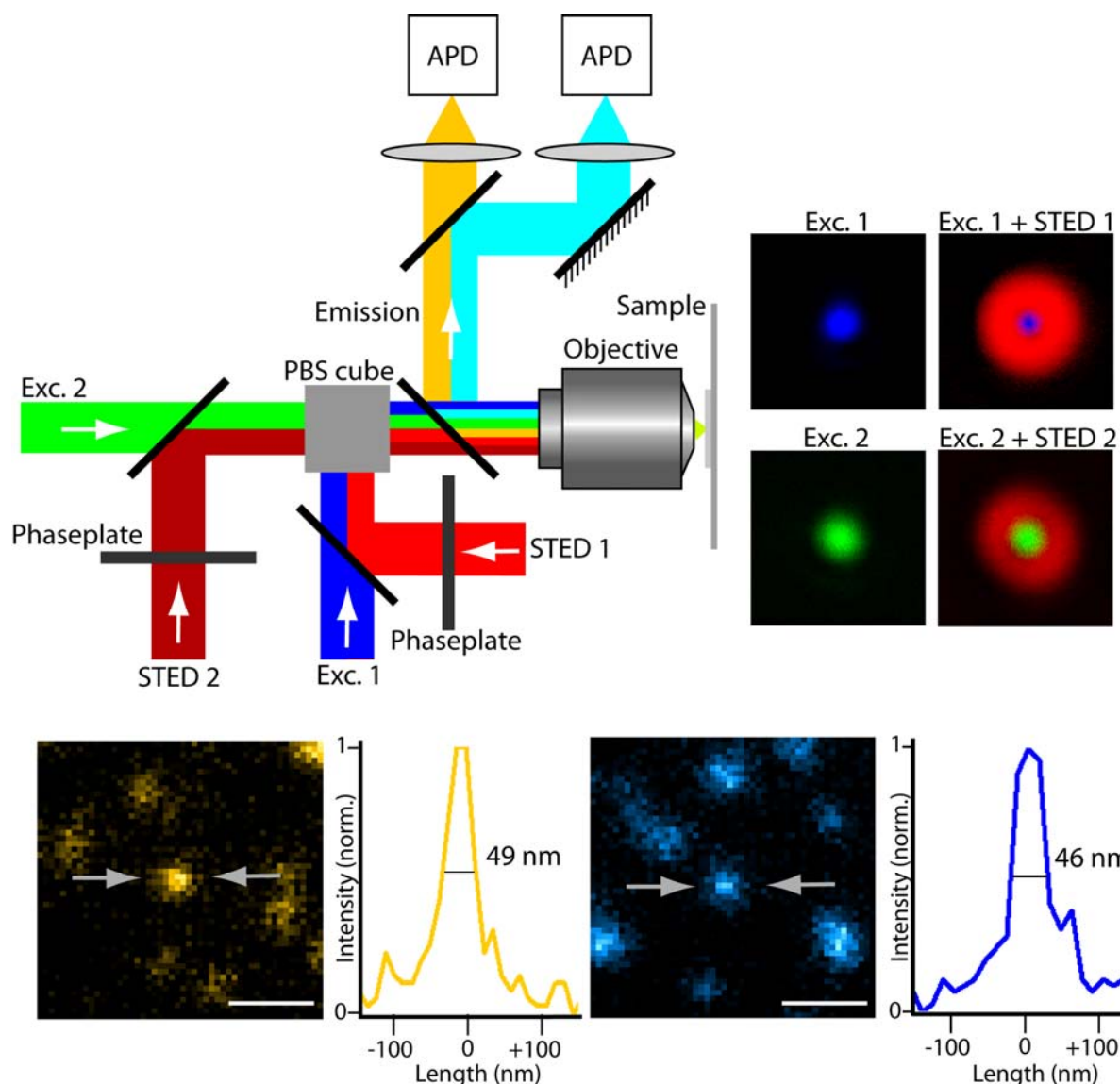


Figure 2: Principal drawing of the two-colour STED setup in Stockholm. From the emission of one and the same supercontinuum laser, four different, spectrally separated beams are obtained and fed into the sample close to the microscope objective. Two of the beams (Exc.1 and Exc.2) specifically excite two different fluorophores in the sample, the other two beams (STED 1 and STED 2) perform light quenching by stimulated emission on the fluorophores excited by the excitation beams, but only in the peripheral range of the excitation cross section area (upper right). Thereby, the volume from which fluorescence is emitted is confined. It is this confinement of the fluorescence emission volume which is the key to the resolution enhancement. The more this volume can be confined, the higher the resolution can get. In the setup, the excitation and STED laser beams are delivered by optical fibres, are collimated and combined pair-wise using dichroic mirrors (mirrors with wave-length specific transmission and reflection). The beam pairs are combined with a polarising beam splitter cube and subsequently fed into the objective lens. Two vortex phase filters induce doughnut-shaped STED beams which confine the fluorescence emission to sub-diffraction-sized region around the focal point (upper right). Bottom: Images of 20 nm and 40 nm fluorescent beads measured in the two different spectral ranges. Taken into account the physical size of these beads, resolution well below 40 nm is achieved in both channels.

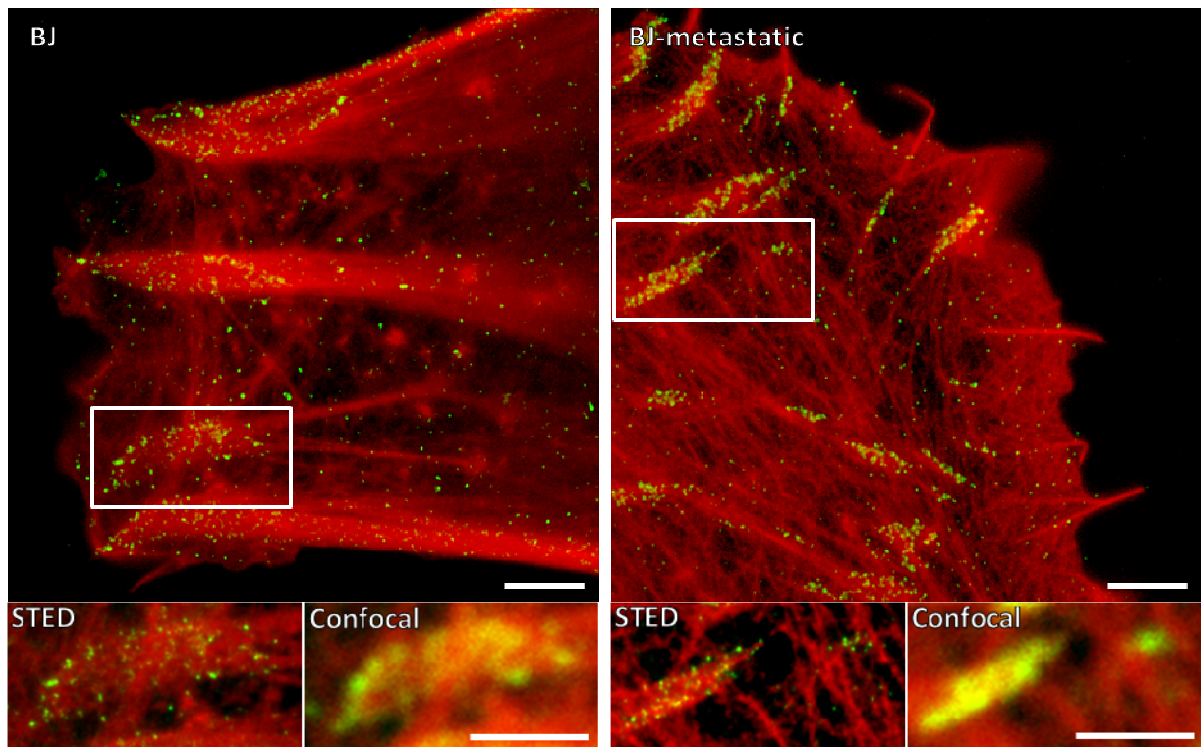


Figure 3: Adhesion spots (green) and the cytoskeletal protein actin (red) images on fibroblast cells representing normal control (BJ, left) and metastatic (BJ-metastatic, right) cells. Scale bars 2 μm . Small images below show the difference in resolving power between STED and conventional confocal microscopy. Via analysis of the sizes, numbers and densities of the adhesion spots clear differences between the control and the malignant cells could be quantified. Likewise, differences in the thickness and densities of the actin filaments could be discerned.

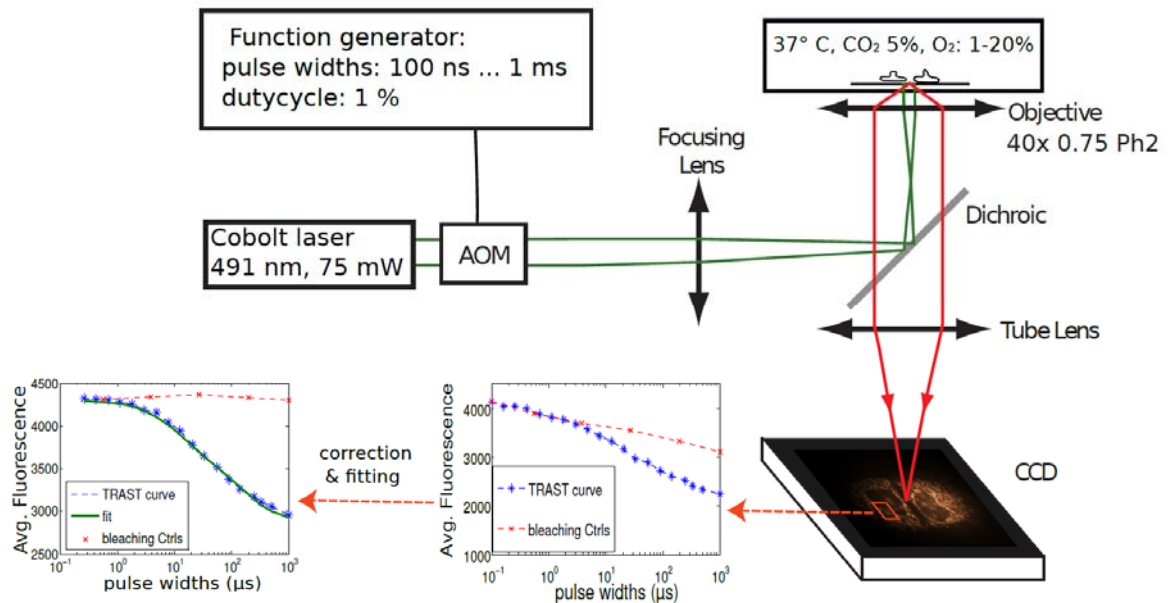
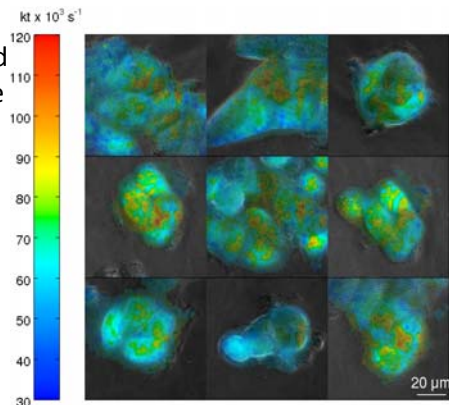


Figure 4: Top: Experimental setup for TRAST microscopy, based on a wide-field microscopy arrangement. A laser is fed into the microscope for excitation, and is modulated in time by an acousto-optical modulator (AOM). The characteristics of the laser modulation, in particular the duration of the excitation periods (pulse widths), influence the average population of transient states of the fluorophores in the sample and thus also the fluorescence intensity the fluorophores emit. By applying pulse trains with different pulse widths, intervals between pulses and/or pulse heights the recorded average fluorescence intensity will change in a systematic way (variation of fluorescence with pulse width shown in graphs above). From this systematic variation of the time-averaged fluorescence intensity the transient state kinetic rates can be determined. Right: Set of images recorded by the instrumentation above, showing the triplet state decay time of the dye Eosin. The triplet state decay time is proportional to the oxygen concentration, and thus the images show maps of the oxygen concentrations (which in turn reflect the local oxygen consumption in the cells).



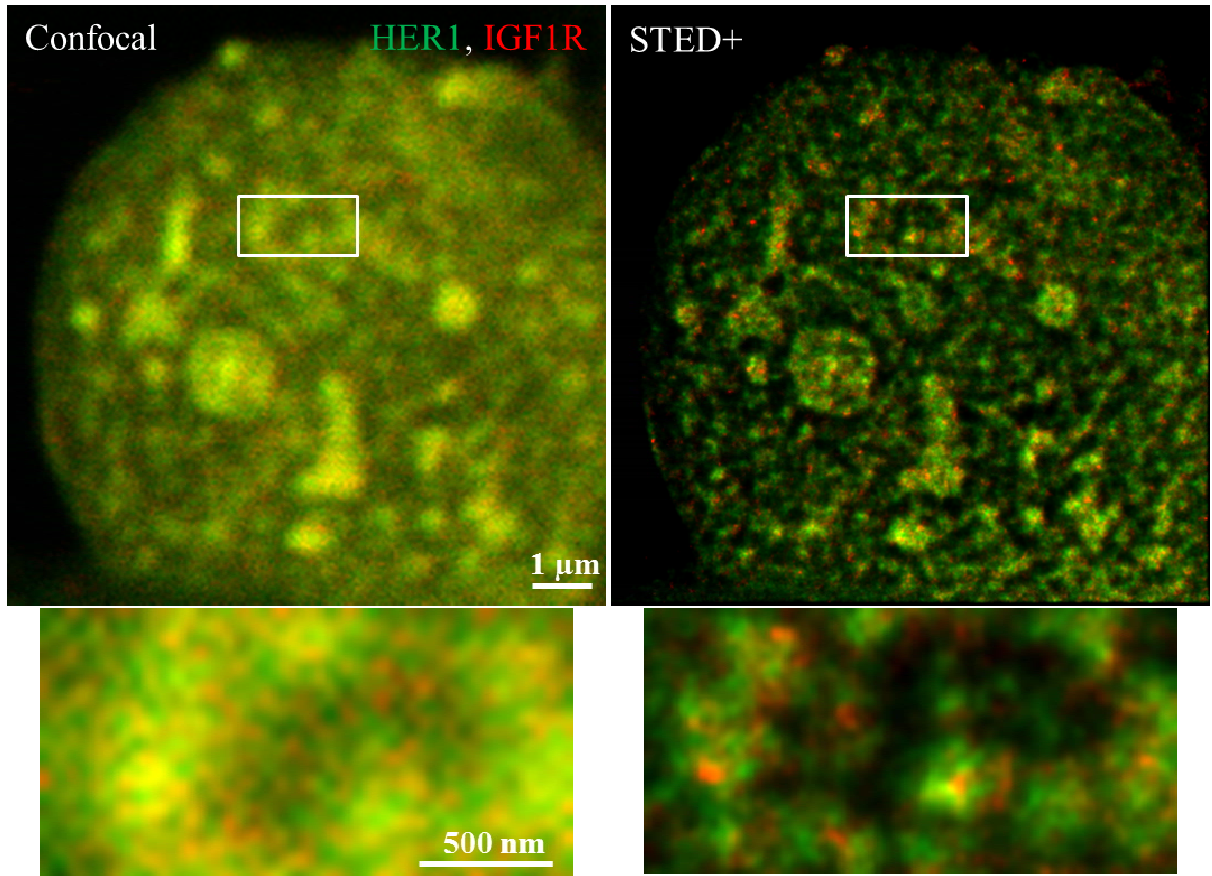


Figure 7: Confocal vs STED images of an FNA-sampled cell from the breast, showing the distribution of HER1 (green) and IGF1R (red). Note the difference in resolution.

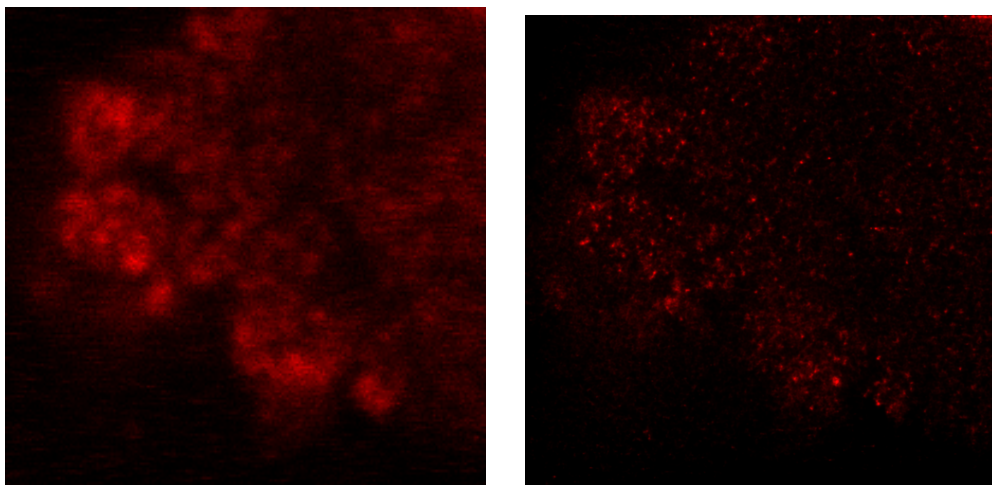


Figure 8: Confocal (left) and nanoscopy (right) images of IGF1R in a cell from a prostate cancer FNA sample.

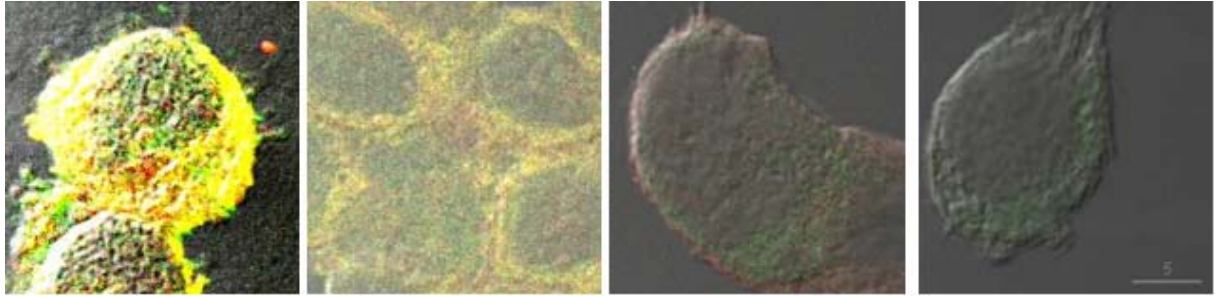


Figure 9: Merged confocal and differential interference contrast (DIC, an optical microscopy illumination technique used to enhance the contrast in unstained, transparent samples based on differences in refraction indices within the sample) images of FNA sampled cells. For the MFDi imaging at HHU and for each patient, one sample was stained for six tumour biomarkers in parallel (left), one sample was stained using only three spectrally fully resolvable affibodies (centre left), one sample was used to monitor unspecific binding by incubating only with secondary antibodies (centre right) and one sample was imaged unstained to analyse autofluorescence.