


New Insights into Cell/Tissue Function with Phosphorescent Oxygen Sensing Probes

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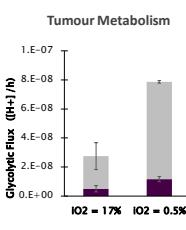


Outline of the Talk

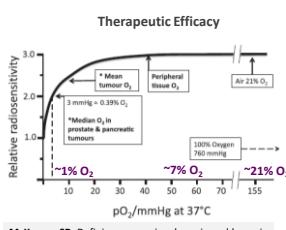
- ▶ Biological Roles of O₂ and principles of quenched-phosphorescence O₂ sensing
- ▶ Demonstration in *in vivo* studies
- ▶ Demonstration in *ex-vivo* models
- ▶ 3D tissue models and physiological studies *in vitro*

Oxygenation and (Tumour) Biology

Tumour Metabolism



Therapeutic Efficacy

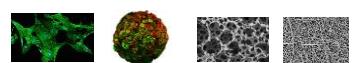


McKeown SR. Defining normoxia, physoxia and hypoxia in tumours—implications for treatment response.
 Br J Radiol 2014;87:20130676

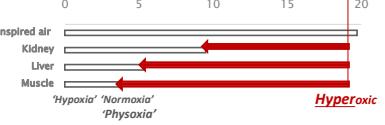
- Therapeutic Efficacy
- Delineating hypoxia signalling (HIF stabilisation)
- Ischemia Reperfusion Modelling

Oxygenation and Physiological Relevance

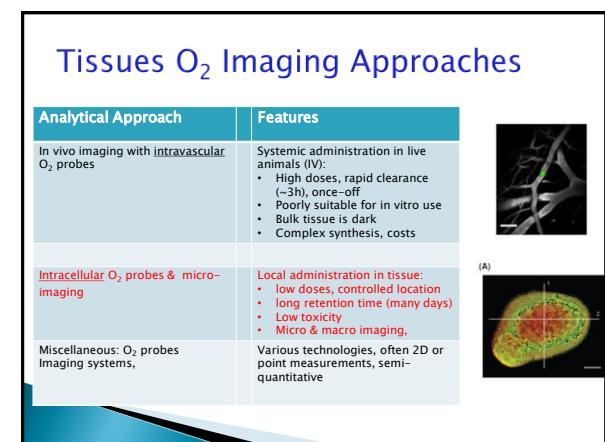
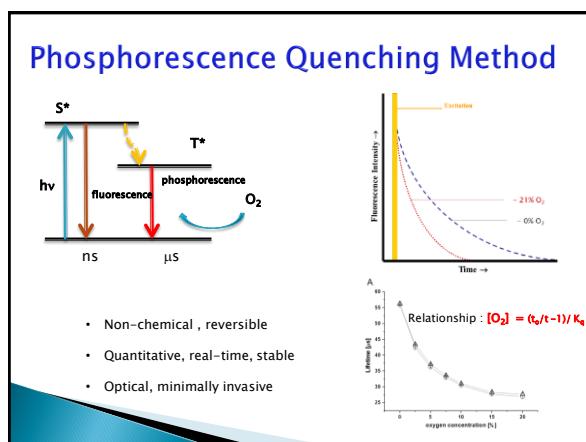
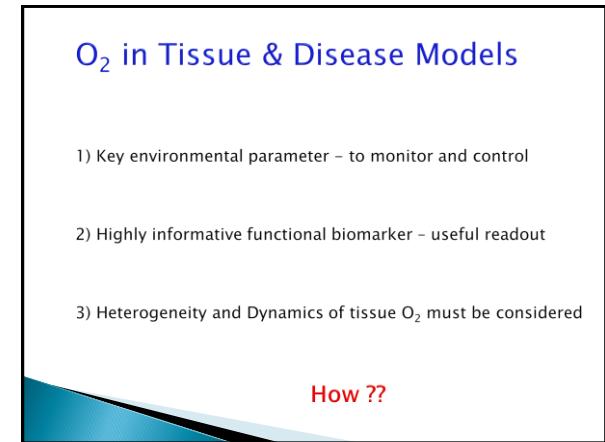
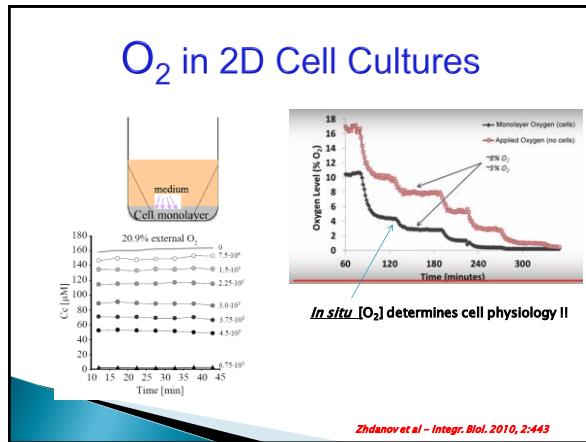
Cell Models & 3D culture



Oxygen



Q) Is culturing cells at 18.6% O₂ physiologically relevant? **A) No.** It is **Hyperoxic** w.r.t. to most tissues.
 ROS / Metabolism / Signalling?



Intracellular O₂ Probes

- Small molecules
- Bioconjugates, e.g. peptides
- Polymeric NPs (by inclusion)
- Polymeric NPs (by conjugation)
- Multi-functional NP composites

O₂ Probe Structures

Probe name, composition	λ_{exc}	λ_{em}	τ_c (μs)	K_{SV}
Ru(bpy) ₃ ²⁺ - CPP conjugate	458 nm	610 nm	775 ns	ND
Ir - CPP conjugate	390 nm	650 nm	50-70 μs	0.0005 mM ⁻¹
Ir - BTP coumarin C343 conjugate	405 nm	620, 680 nm	5.6 μs	0.004 mM Hg ⁻¹
Ir(CP) - CPP complexes	386	654	58-69 μs	0.074 mM ⁻¹
PTTFPP-Pt-Cy conjugate	395	650	57 μs	0.03 mM ⁻¹
PdTCBP-HLyte650 dendrimer in PAAC NPs modified with TAT peptide (30-50 nm)	442, 632 nm	790 nm (O ₂) 699 nm (ref)	Not reported (250 μs for G2)	0.034 mM ⁻¹
[Ru(dppz(Na ₂)) ₃]Cl in PAA NPs (45 nm)	454 nm	608 nm	3.88-4.06 μs	ND
PTTFPP in RL100 polymer (33 nm NPs)	395 nm	650 nm	69.1 μs	0.04 mM ⁻¹
PTTFPP-naphthalimide dye in PS NPs (410-430 nm)	395 nm	650 nm 490 nm (ref)	ND	ND
PTTFPP and PFO in RL100 NPs (70 nm)	405 nm - 1P 760 nm - 2P	650 nm 430 nm (ref)	66 μs	0.041 mM ⁻¹
[Ru(dppz) ₃]Cl/TMSPS in amino modified PS NPs (121 nm)	488 - 1P 830 nm - 2P	630 nm	ND	-0.87
PTTFPP in KU100 NPs	440, 614 nm	700 nm	57 μs	-0.02 mM ⁻¹
PTTFPP in PS NPs (50 nm)	395	650	61 μs	ND
PTTFPP and PFO in acrylic polymer NPs (95 nm)	405 - 1P 760 - 2P	650 nm 430 nm (ref)	68 μs	0.086 mM ⁻¹
WPF-I4 and WPF-Ir6 NPs (19 nm)	405 nm	630 nm 450 nm (ref)	0.6 μs	0.006 mm Hg ⁻¹ ?
[Ru(dppz) ₃]Cl ₂ + NaYF ₆ :Yb/Tm//NaYF ₄ in mesoporous silica NP (50 nm)	980 nm - UC	613 nm 477 nm (ref)	ns	ND
PTTFPP in PS-silane hybrid NPs (77 nm)	395	605 nm	Ms	ND

Nanoparticle Probes: NanO₂

- Biocompatible polymer
- Average size 35-50 nm
- Z potential +45mV
- Stable, bright, low toxicity

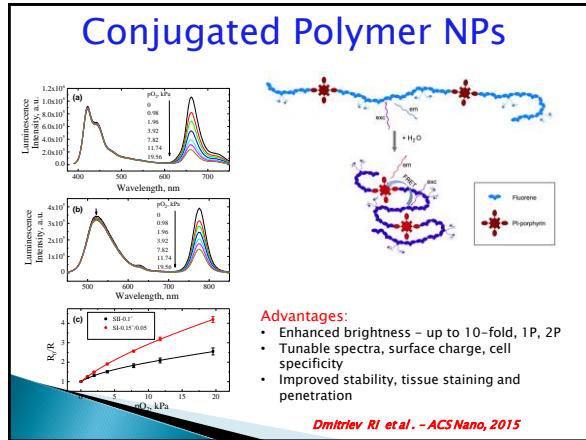
Detection Modes:

Probe	Excitation, nm	Emission	Readout Signal
NanO ₂	390-405 nm	650 nm	Lifetime (μs FLIM)
	390-405 nm	650 nm	Intensity

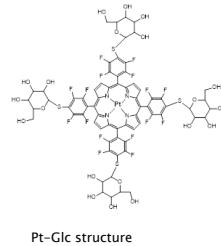
Fercher A. et al - ACS Nano, 2011

Mitolimage™ Probe Family

Dmitriev RI et al. - Adv. Funct. Mater 2012



Small Molecule Pt-Glc Probe



- Hydrophilic, water-soluble, neutral
- Efficient cell and tissue penetration
- Stable calibration
- High photostability, low toxicity
- Moderate brightness

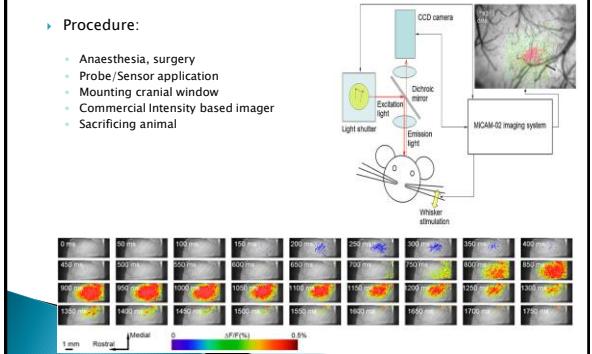
Dmitriev RI et al. - Biomater. Sci., 2014

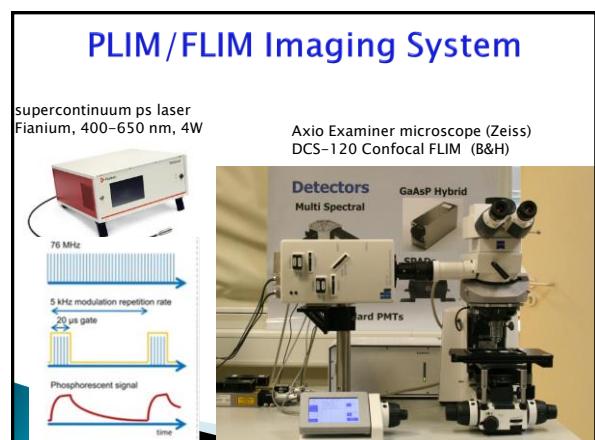
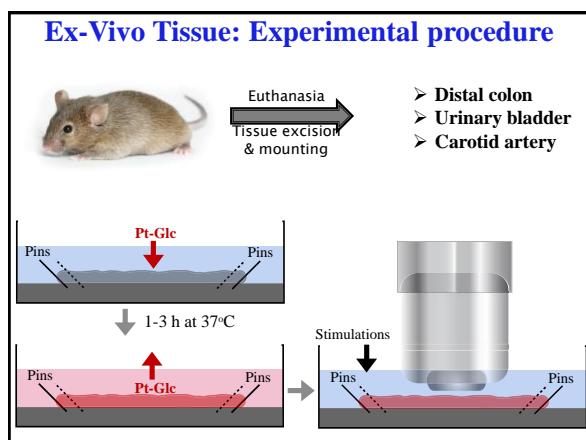
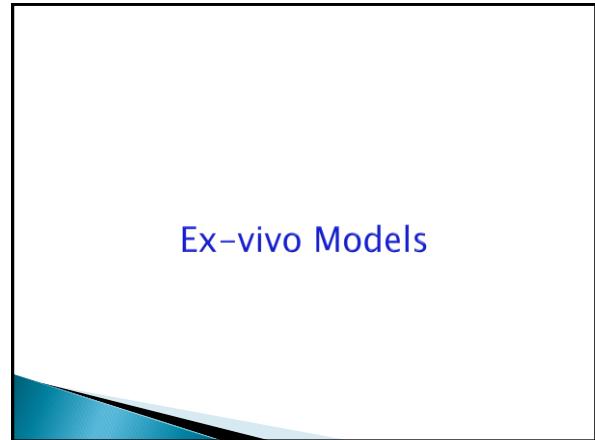
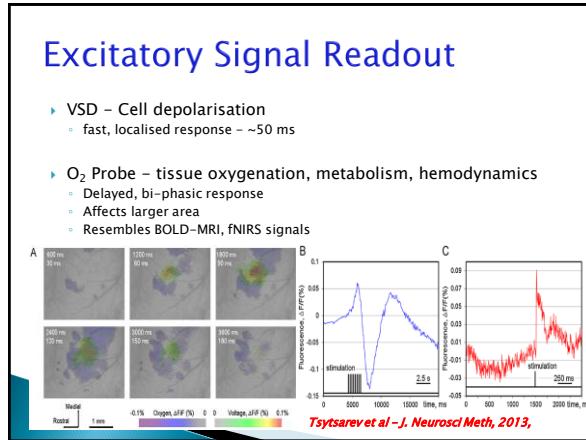
In vivo Models

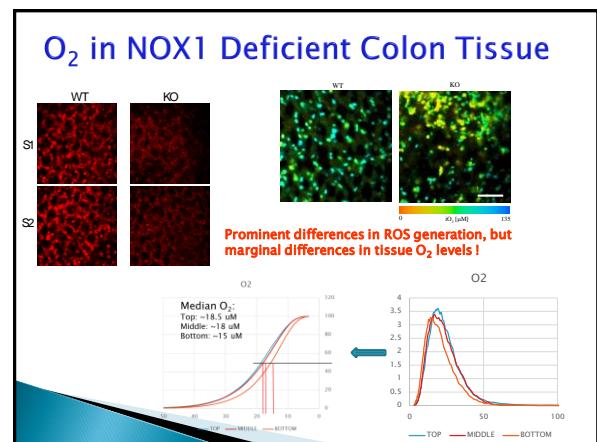
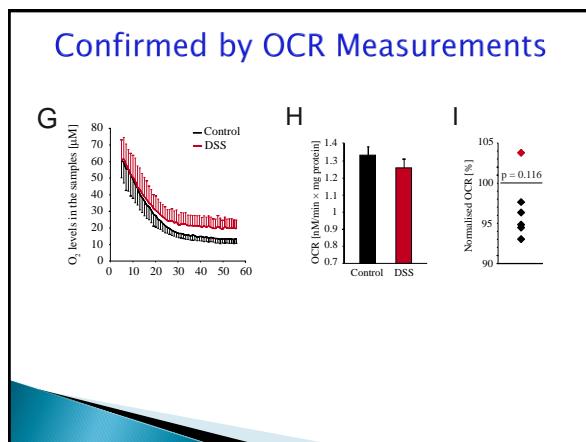
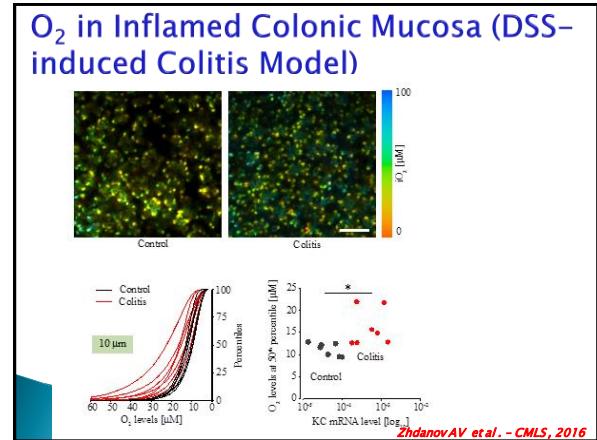
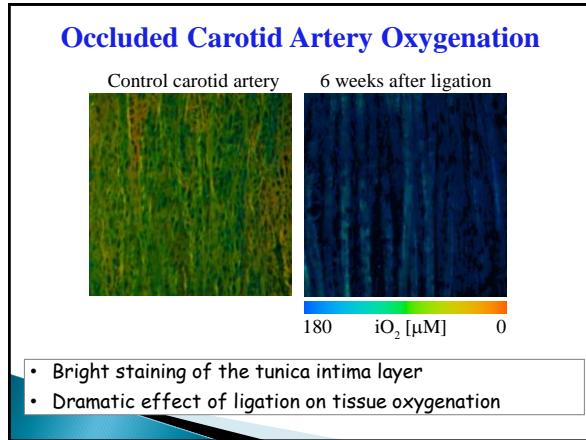
Functional Brain Imaging

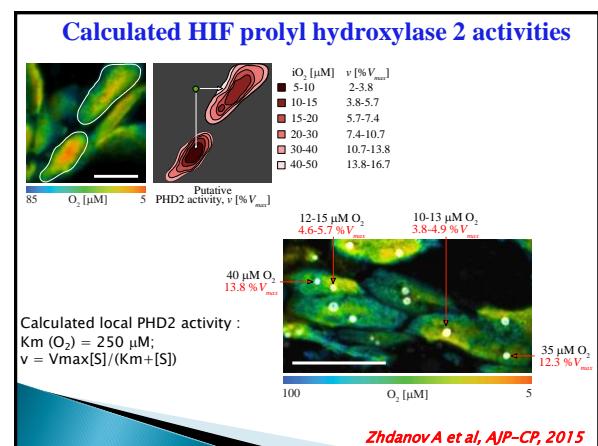
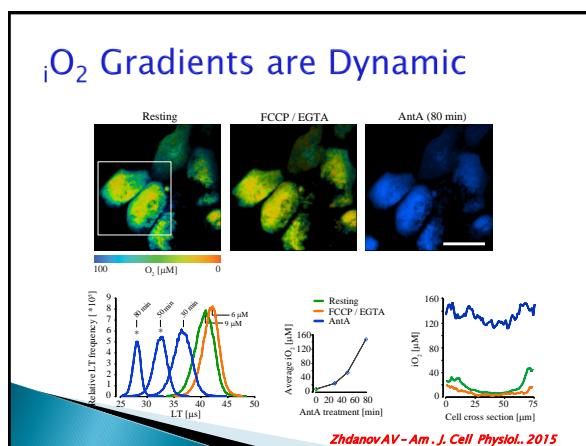
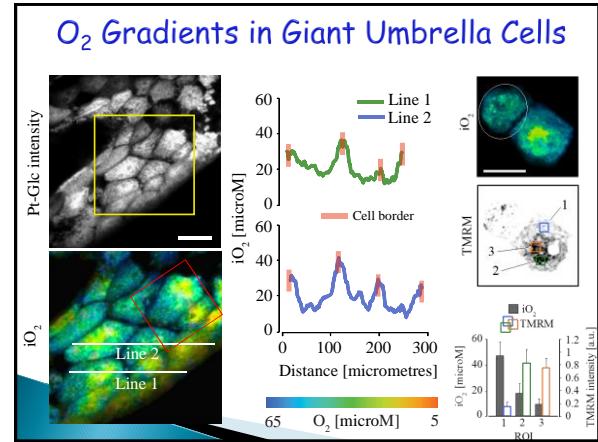
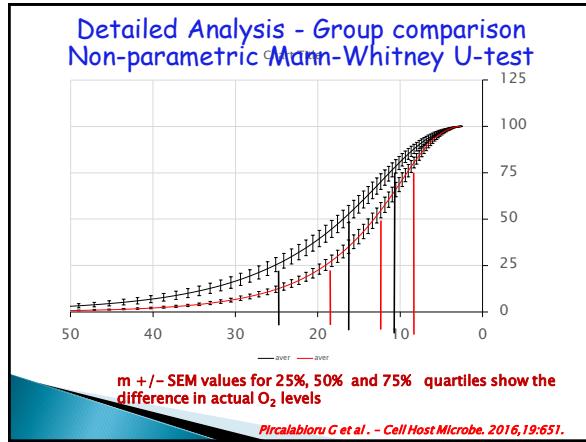
Procedure:

- Anaesthesia, surgery
- Probe/Sensor application
- Mounting cranial window
- Commercial Intensity based imager
- Sacrificing animal





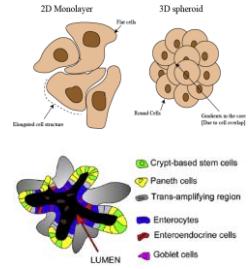




In vitro Tissue Models

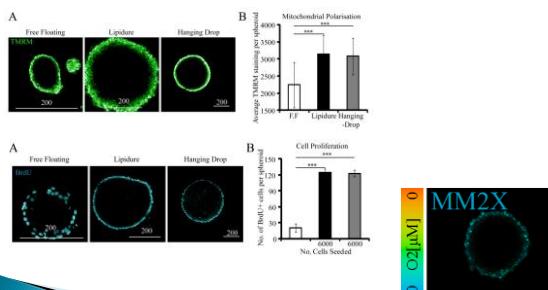
Common Models

- Multicellular spheroids
- Cell co-cultures
- Organoids
- Engineered tissue scaffolds
- Vascularised tissue

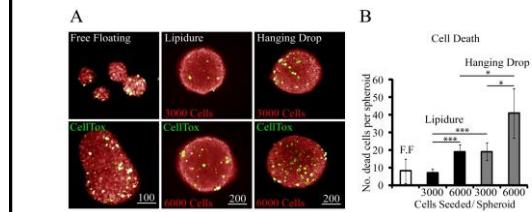


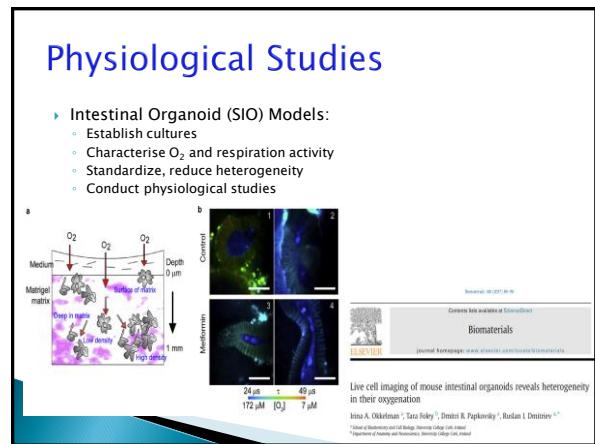
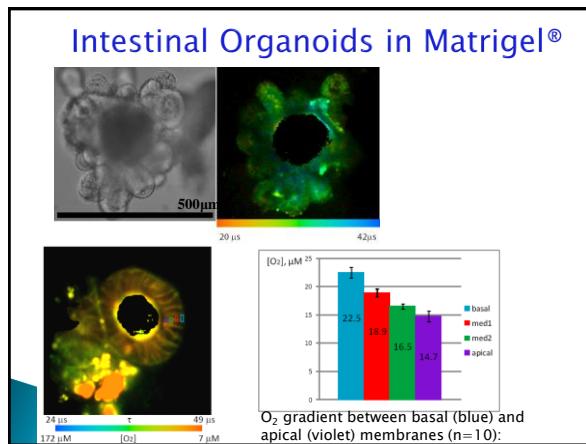
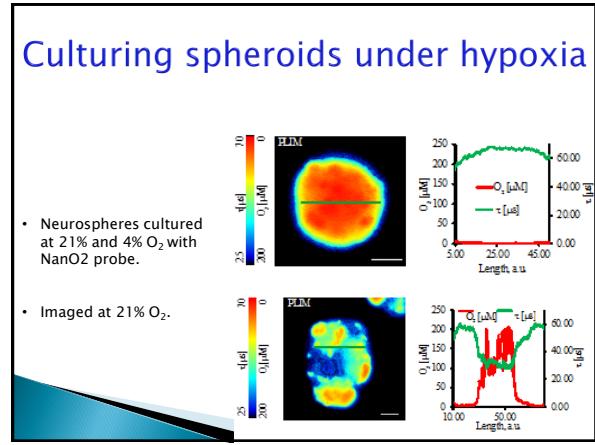
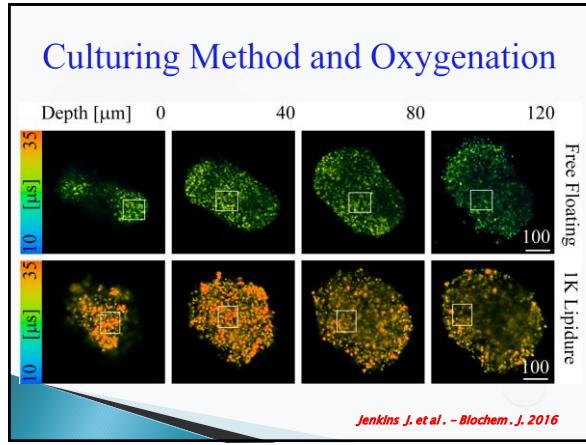
Environmental control and standardization remain bottlenecks
FLIM platforms can address these

Challenges – Tissue Staining



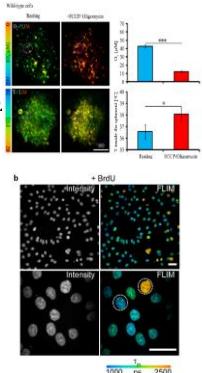
Pre-Staining with O₂ probes





Other FLIM Probes & Assays

- ▶ T - probe (NanO₂ analog) - *Anal Chem*, 2016, 88: 10566
- ▶ pH probe - *J. Mater. Chem. B*, 2014, 2: 679.
- ▶ Cell Cycle assay (Hoechst 2334 and dBrU) - *PLOS One*, 2016, 11: e0167385
- ▶ K⁺-probe - *Adv Funct Mater* (in press)
- ▶ More in development



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