August 25, 2017

Dear All: I recently sent around discussions of ways to measure/estimate drug concentrations both in living animals (aptamer-based probes, Kevin Plaxco's group) and with sufficient spatial resolution to permit construction of 2d/3d drug concentration maps (MALDI-MSI, David Perlin's group). These notes generated a lot of interest and in particular Ed Weinstein (FDA) made me aware of a book chapter in which he and his co-authors had looked at a range of such techniques.

Finding this intriguing, I worked with Kevin, David, and Ed to prepare a summary of the range of current techniques for doing something that you could loosely call PK in high resolution by time and/or space. Several ways of categorizing the methods are shown in the table below.. It's fascinating to see them side-by-side and realize the wealth of opportunities that exist.

Each approach has strengths and weaknesses but I must say that MALDI-MSI and aptamer-based probes really standout as exciting new tools by offering **strong spatial information** (MALDI-MSI) or **easily obtained continuous drug measures** (aptamer-based probes) **based in both cases on measurements of the unmodified analyte**.

Overview of techniques: Specialized probe and/or modified analyte vs. general approach to detection of unmodified analytes

	Continuous, with potential for in vivo	Single-time point*
Spatial resolution	• Fluorescent probe • Hyperpolarized NMR/MRI • PET Imaging • Photoacoustic imaging	• Low energy (^{3}H or ^{14}C) radio-labeled analyte (Whole-Body Autoradiography) • MALDI-MSI • Laser-capture microdissection
Specific site	• Aptamers on a probe • Microdialysis	• Grind tissue & assay extract

*Of course, any single-time point method could be made effectively continuous by doing serial sampling but there are practical limits to how much of this you can do.

Rough rank order of spatial imaging resolution methods:

1) Higher resolution: MALDI-MSI, autoradiography
2) Lower resolution: Fluorescent probe, Hyperpolarized NMR/MRI, PET Imaging, Photoacoustic imaging, Laser-capture microdissection

Absolute analyte concentration data: (1) Aptamers on a probe, (2) Microdialysis (with difficulty), (3) Laser-capture microdissection, and (4) Grind tissue & assay extract

If this intrigues, you may also want to look both this book and PK-specific chapter within it:

1) Book: Imaging Infections : From Bench to Bedside. S. K. Jain.: https://link.springer.com/book/10.1007%2F978-3-319-54592-9
2) Chapter in said book Ordonez, A. A., L. E. Bambarger, S. K. Jain and E. A. Weinstein (2017). Biodistribution and Pharmacokinetics of Antimicrobials. Imaging Infections : From Bench to Bedside. S. K. Jain. Cham, Springer International Publishing**:** 209-222. :https://link.springer.com/chapter/10.1007/978-3-319-54592-9_10

And while you are exercising your PK-PD neurons, you might also want to review the material from the 14-15 June 2017 NIAID PK-PD workshop, the excellent EMA guideline on PK-PD for antibacterials, and the discussions of PK-PD at the 1 Mar 2017 FDA workshop on animal models in support of narrow-spectrum agents for *A. baumannii* and *P. aeruginosa*.

All best wishes **and with thanks to Ed, David, and Kevin** for provoking this interesting exploration, --jr

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Brief summary of techniques for high-resolution (time or space) PK

Method	Short summary	Pro/Co	Examples with links
Aptamers on a probe	Surface bound aptamer binds analyte. Changes in aptamer configuration is sensed electronically.	1) Pro: Real-time, continuous, can be in vivo 2) Pro: Absolute concentrations can be determined 3) Con: Must create a suitable aptamer for each analyte 4) Con: Sensor is one place - no spatial resolution	In vivo: Arroyo-Curras, N., J. Somerson, P. A. Vieira, K. L. Ploense, T. E. Kippin and K. W. Plaxco (2017). "Real-time measurement of small molecules directly in awake, ambulatory animals." Proc Natl Acad Sci U S A 114(4): 645-650. Open-Access. http://amr.solutions/blog/real-time-continuous-in-vivo-drug-monitoring plus this white paper on Arroyo-Curras' method. Non-in vivo: Wiedman, G. R., Y. Zhao, A. Mustaev, J. Ping, R. Vishnubhotla, A. T. C. Johnson and D. S. Perlin (2017). "An Aptamer-Based Biosensor for the Azole Class of Antifungal Drugs." mSphere 2(4): 1-10. Open access. http://msphere.asm.org/content/2/4/e00274-17
Fluorescent probe https://en.wikipedia.org/wiki/Chemical_imaging	Fluorescent probe is bound to a macromolecule such as an antibody	1) Pro: Real-time, continuous, can be in vivo 2) Pro: Provides spatial resolution 3) Con: Requires chemical modification of the parent drug which may alter its properties 4) Con: Best for larger molecules as the size of the probe strongly alters the behavior of smaller molecules 5) Con: Can't see very deeply. Light has limited tissue penetration due absorption.	Cilliers, C., I. Nessler, N. Christodolu and G. M. Thurber (2017). "Tracking Antibody Distribution with Near-Infrared Fluorescent Dyes: Impact of Dye Structure and Degree of Labeling on Plasma Clearance." Mol Pharm **14**(5): 1623-1633. Open access. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5415873/pdf/mp6b01091.pdf

Method	Short summary	Pro/Co	Examples with links
Grind tissue and assay extract	After drug administration, whole organs are ground and analyte is extracted	1) Pro: Well-established, no special equipment 2) Pro: Works for any analyte and gives absolute concentrations 3) Con: Single time-point 4) Con: No spatial resolution	Widely used – many published examples
Hyperpolarized NMR/MRI https://en.wikipedia.org/wiki/Hyperpolarization_(physics)	Isotopically labeled probe molecules with hyperpolarized protons are injected and metabolic conversion can be tracked	1) Pro: Real-time, continuous, can be in vivo 2) Pro: Provides spatial resolution 3) Con: Requires isotopically labeled probe 4) Con: Probe is short-lived (few minute duration at best)	Meier, S., P. R. Jensen, M. Karlsson and M. H. Lerche (2014). "Hyperpolarized NMR probes for biological assays." Sensors (Basel) 14(1): 1576-1597. Open access https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3926627/
Laser-capture microdissection	Optical laser-based dissection of tissue fragments which are captured for analysis	1) Pro: Provides spatial resolution within limits of dissection resolution 2) Pro: Works for any analyte and gives absolute concentrations 3) Con: Single time-point 4) Con: Tedious for extensive spatial mapping	Good discussion at https://en.wikipedia.org/wiki/Laser_capture_microdissection. Example of use: Zhao, Y., B. Prideaux, Y. Nagasaki, M. H. Lee, P. Y. Chen, L. Blanc, H. Ho, C. J. Clancy, M. H. Nguyen, V. Dartois and D. S. Perlin (2017). "Unraveling Drug Penetration of Echinocandin Antifungals at the Site of Infection in an Intra-Abdominal Abscess Model." Antimicrob Agents Chemother.
Low energy (^{3}H or ^{14}C) radio-labeled analyte (Whole-Body Autoradiography)	Labeled analyte is visualized by autoradiography of tissue slide	5) Pro: Provides spatial resolution 6) Con: Not absolute: only relative drug concentrations can be estimated 7) Con: Single time point	Widely used – many published examples

Method	Short summary	Pro/Co	Examples with links
MALDI MSI	2D (x-y) MALDI scan of tissue slide	1) Pro: Spatial mapping 2) Pro: Any analyte, including metabolites 3) Con: Not absolute: only relative drug concentrations can be estimated 4) Con: Single time point	Tracking a drug: Zhao, Y., B. Prideaux, Y. Nagasaki, M. H. Lee, P. Y. Chen, L. Blanc, H. Ho, C. J. Clancy, M. H. Nguyen, V. Dartois and D. S. Perlin (2017). "Unraveling Drug Penetration of Echinocandin Antifungals at the Site of Infection in an Intra-Abdominal Abscess Model." Antimicrob Agents Chemother. Open Access. See also: http://amr.solutions/blog/high-resolution-effectively-3-d-drug-concentration-measurements-in-tissues Tracking other multiple analytes: Scott, A. J., J. W. Jones, C. M. Orschell, T. J. MacVittie, M. A. Kane and R. K. Ernst (2014). "Mass Spectrometry Imaging Enriches Biomarker Discovery Approaches with Candidate Mapping." Health Physics **106**(1): 120-128. https://www.ncbi.nlm.nih.gov/pubmed/24276555
Microdialysis https://en.wikipedia.org/wiki/Microdialysis	Tiny probe, push dialysate fluid through, capture analyte for later analysis	1) Pro: Well established 2) Pro: Can detect any analyte 3) Pro: Can be semi-continuous but isn't real-time as samples must be analyzed 4) Con: Not spatial … probe is where it is. 5) Con: Not quantitative without cumbersome determination of dilution and recovery efficiencies.	de Araujo, B. V., A. Diniz, E. C. Palma, C. Buffe and T. Dalla Costa (2011). "PK-PD modeling of beta-lactam antibiotics: in vitro or in vivo models?" J Antibiot (Tokyo) 64(6): 439-446. Open Access. http://www.nature.com/ja/journal/v64/n6/full/ja201129a.html?foxtrotcallback=true

Method	Short summary	Pro/Co	Examples with links
PET Imaging https://en.wikipedia.org/wiki/Positron_emission_tomography	Gamma rays from positron emitting tracer is detected	1) Pro: Real-time, continuous, can be in vivo 2) Pro: Provides spatial resolution 3) Con: Requires an isotopically labeled molecule. Synthetic radiochemistry can be a challenge. 4) Con: Duration limited by short half lives of minutes or a few hours.	DeMarco, V. P., A. A. Ordonez, M. Klunk, B. Prideaux, H. Wang, Z. Zhuo, P. J. Tonge, R. F. Dannals, D. P. Holt, C. K. Lee, E. A. Weinstein, V. Dartois, K. E. Dooley and S. K. Jain (2015). "Determination of [11C]rifampin pharmacokinetics within Mycobacterium tuberculosis-infected mice by using dynamic positron emission tomography bioimaging." Antimicrob Agents Chemother 59(9): 5768-5774. Open Access. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4538528/
Photoacoustic imaging https://en.wikipedia.org/wiki/Photoacoustic_effect	Absorption of light causes production of heat that becomes sound that is detected by (e.g.) piezoelectric sensors. Change in state of a chromoionophore on binding of an analyte is measured by differences in acoustic signature.	1) Pro: Real-time, continuous, can be in vivo 2) Pro: Provides spatial resolution 3) Con: Can't see very deeply into tissues 4) Must be able to design a suitable chromoionophore.	Cash, K. J., C. Li, J. Xia, L. V. Wang and H. A. Clark (2015). "Optical drug monitoring: photoacoustic imaging of nanosensors to monitor therapeutic lithium in vivo." ACS Nano 9(2): 1692-1698. Not Open Access. http://pubs.acs.org/doi/pdf/10.1021/nn5064858