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Top-Down Ramanomics Instrumentation Overview: from Quantitative Ramanomics with Deep Convolutional Neural Networks for Intraoperative Point-of-Care Testing Applications to Molecular Optical Laser Examiners. Part I (Bibliographic Review)

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Abstract

This review paper provides a retrospective analysis of ranomics technologies and their methodological predecessors, ranging from modern quantitative ranomics using deep convolutional neural networks (used for intraoperative and point-of-care diagnostics) to the Molecular Optical Laser Examiners (MOLE) of the 1970s. The first part of the review examines the current directions of this trend, while the second part presents the achievements of the earlier period. The first review part pays the special attention to applications of ramanomics for diagnostics of "supramolecular pathologies", mechanisms of apoptosis, parabiosis, oncogenesis, redox pathologies (as well as effects of active oxygen species on cells and tissues), damages of the blood-brain barrier and neurotraumas affecting the cytoarchitectonics of the brain (or, more broadly, the architecture of neuronal connectomes). A number of works are indicated that allow us to speak about Raman analysis for spectral comparative pathological organellography of the cytoplasm. Also information is given on the integrability of ramanomics with methods of mass-spectrometric mapping of biomedical samples (i.e. RaMALDI), including for MALDI-biotyping tasks for clinical microbiology applications.

Keywords: ramanomics, qRamanomics, spectralomics, single-organelle optical omics, MALDI MS imaging, RaMALDI, simultaneous Raman and MALDI imaging, label-free time-resolved single-cell monitoring, intraoperative diagnostics; point-of-care diagnostics, convolutional neural networks.

1. Введение

Что такое рамаомика?

Принципиально новым омиксным направлением в молекулярной и клеточной биологии и техническим прорывом в области биомолекулярной спектроскопии является рамаомика (Kuzmin et al., 2017a). По определению из только что цитированной работы, "рамаомика – … омиксная дисциплина, использующие рамановскую микроспектрометрию при анализе биомолекулярных компонентов (в целях) молекулярного профилирования биологических структур". Аналогичное определение было институционализировано на "2nd International Symposium on Physics, Engineering and Technologies for Biomedicine" ("...Ramanomics which is a new... disciplines using Micro Raman Spectrometry with Biomolecular Component Analysis for molecular profiling of biological structures"). В настоящее время данная

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дисциплина заняла прочное место в кругу омиксных дисциплин и спектральных методов химического картирования клеток, рассматриваясь как одно из наиболее сильных и многообещающих достижений инструментальной оптики и фотоники в областях рамановской спектрометрии и биомедицинского имэджинга (Siddhanta et al., 2023).

На рубеже последних лет она перешла из разряда методов качественного имэджинга (imaging) и оценивания (estimation) распределения спектрометрируемых биомолекул в плоскости микропрепарата (нередко – фиксированного) к количественному анализу 3D распределений соответствующих биомолекул, в том числе – в ходе отклика на какие-то фармацевтические или токси(колог)ческие воздействия. Такое направление называется "quantitative Ramanomics" – "qRamanomics" (LaLone et al., 2023; Dunnington et al., 2024). Для него подходит не только обычная рамановская микроспектрометрия, но и методики спектроскопии поверхностно-усиленного комбинационного рассеяния (surface-enhanced Raman spectroscopy) субклеточного уровня разрешения (Zhang et al., 2019; Shen et al., 2021). Рамановика как способ фенотипирования и профилирования клеток позволяет выявить (для последующей идентификации и прогностики) т.н. "рамановский фенотип" (Zhang et al., 2019) – частный случай "расширенного фенотипа" или морфометрии клетки по невизуальным критериям (Jablokov, Gradov, 2016). Это важно, в частности, для разработки методов рамановской спектральной цитометрии (LaLone et al., 2019a; LaLone et al., 2019b) и оперативного (point-of-care) интраоперационного рамановского контроля метаболома пациента (DePaoli et al., 2020; Huang et al., 2023).

Рамановика позволяет регистрировать и идентифицировать спектры одиночных органелл (как "single-organelle optical omics" (Pliss et al., 2021)). Собственно, в наиболее импактном изложении данного подхода (Kuzmin et al., 2017b) акцент в названии ("Molecular profiling of single organelles for quantitative analysis of cellular heterogeneity") делался на построении карт гетерогенности клеточных структур, т.е. молекулярном профилировании органелл. А в наиболее свежем обзоре на эту тему, интегрирующем разные методы вибрационной микроспектроскопии- как рамановской, так и инфракрасной – в целях безметочного (label-free) определения и времяразрешенного мониторинга биохимических конституентов клетки, акцент в дефиниции термина был сделан на субклеточном уровне ("Ramanomics has been used previously to infer the use of the technique to analyse and monitor the biochemical constituent content *at a subcellular level*") (Byrne, 2024). Речь идёт, в частности, о биомембранах и мембранных органеллах (как плазматической мембране клетки и мембранах органелл типа митохондрий, так и внеклеточных везикулах (Guerreiro et al., 2024)), а также идентификации примембранных и мембранных белков и кинетической идентификации их изменений в физиологических и цитопатологических процессах (Tian et al., 2021). Последнее направление также весьма эффективно реализуется с использованием спектроскопии поверхностно-усиленного комбинационного рассеяния (surface-enhanced Raman spectroscopy) субклеточного уровня разрешения, процитированных выше (Zhang et al., 2019; Shen et al., 2021).

Это важно для выявления клеточных патологий (конечно, не в трактовке "целлюлярной патологии" Р. Вирхова XIX века, но в также классической трактовке "молекулярной" или "супрамолекулярной патологии" (Поликар А., Бесси М., 1970).

Например, в клинической хондриомике апоптоз эндотелиальных клеток сосудов головного мозга после ряда травм может быть связан с дисфункцией пула митохондрий, а его можно идентифицировать по рамановским микроспектрам – следовательно, ряд повреждений гематоэнцефалического барьера (4B = BBBB – Blood Brain Barrier Breakdown "is a key driver of traumatic brain injury (TBI)" (Schmitt et al., 2023) может быть идентифицирован на ультраструктурном уровне с использованием локальных рамановских микроспектрометрических измерений (Schmitt et al., 2023) (без привлечения электронной-микроскопии и сравнительно-патологической органеллографии цитоплазмы (Frey-Wyssling, 1965)) – аппаратурой, ПО и БД рамановики. Аналогичное верно для выявления злокачественного перерождения тканей в процессе онкогенеза: митохондриальные изменения, соответствующие злокачественным клеткам с морфологически сложно выявляемыми изменениями, могут быть выявлены аппаратурой и цифровыми "надстройками" рамановики (Gayan et al., 2022) ("надстройка" здесь может трактоваться как "uberbau", без которого концепт метода не достигает цели – аппаратура с различной разрешающей способностью для Рамановской микроспектрометрии известна, как минимум, с 1970-х гг., но без цифровой "надстройки" для глубинного анализа данных они не могли бы дать такую диагностическую определенность; уровня автоматизации XX века не хватило бы для обеспечения мультикритериальной рамановимиксной диагностики).

2. Обсуждение и результаты

Раманомика: мультипараметрическая корреляция, алгоритмы, машинное обучение.

Вполне понятно, что это было бы невозможно без специализированных алгоритмических средств. В уже цитированной работе (Kuzmin et al., 2017b) писали: "Recent developments in Raman spectroscopy instrumentation and data processing algorithms have led to the emergence of Ramanomics". По существу, рамановский микроспектрометр, используемый для работы в области раманомики, представляет собой не просто оптический прибор, а "программно-аппаратный комплекс" с набором спектральных библиотек и молекулярно-биологических баз данных, экспертными системами (как минимум) или более продвинутыми средствами глубинного анализа данных (KDD) и нейросетевой компонентой / машинным обучением, обеспечивающим пополнение баз и всё более точную идентификацию биомолекулярного контента органелл. Например, в работе того же коллектива авторов, что и в публикациях (Kuzmin et al., 2017a; Kuzmin et al., 2017b), опубликованной годом позже (Kuzmin et al., 2018), вводится алгоритм "BCAbox", усовершенствующий спектр возможностей рамановской аппаратуры (микроскопа-микроспектрометра), а в работах начала 2020-х гг. внедряется комплексный подход, основанный на использовании искусственного интеллекта/машинного обучения (Lu et al., 2020; Lawrence, 2023). Например, в только цитированной работе (Lu et al., 2020) используется свёрточная нейронная сеть (ConvNet), в то время как Лоуренс (Lawrence, 2023), критикуя производительность вычислительных подходов, используемых в "классической" раманомике, считает, что простые методы потенциально могут превзойти глубокое обучение. Он справедливо отмечает, что "хотя глубокое обучение является многообещающим, оно не обеспечивает <<квантового скачка>> в производительности" и приводит свой личный опыт, в котором "простой и интерпретируемый" метод логистической регрессии достиг точности ≈ 90,4% менее, чем за минуту, а использование метода случайного леса решений – random decision forest – RDF (алгоритм машинного обучения Бреймана и Катлер, использующий ансамбль решающих деревьев и применимый для задач классификации, регрессии, кластеризации) обеспечило точность 92,6 % менее чем за 10 сек. Этот опыт противопоставляется (автором данного подхода) использованию глубоких сверточных нейронных сетей в классических и наиболее устоявшихся методах раманомики (DCNN – Deep Convolutional Neural Networks), с помощью которых точность около 90% достигается примерно после часа обучения на современном настольном компьютере (Lawrence, 2023). Идеальным решением были бы методы машинного обучения, учитывающие биофизический контекст: "A biophysics aware machine learning method would be more welcome" (там же (Lawrence, 2023)). "Чтобы действительно знать предмет, надо охватить, изучить все его стороны, все связи и "опосредования" – гласит диалектика.

Кроме того, размерность снятых с позиционной чувствительностью данных раманомики в разы превышает как данные обычной спектроскопии, так и двумерные микрофотографии и химические карты. В цитированной статье (Gayan et al., 2022) указывается на возможность использования конфокальных микроскопов, по определению, способных не только фокусироваться в определенных точках ультраструктуры клетки, но и отстраивать трехмерные реконструкции органелл с использованием лазеров с разными длинами волн, из чего (интуитивным образом) выводится целесообразность интеграции конфокальной и рамановской идентификации в клеточной патологии. Так, в цитируемой работе (Gayan et al., 2022) пишут, что так как "раковые митохондрии демонстрируют различные профили по сравнению с нормальными в морфологии, геномном, транскриптомном, протеомном и метаболическом профиле" ("cancerous mitochondria exhibit different profiles compared with normal ones in morphology, genomic, transcriptomic, proteomic and metabolic landscape"), их дифференциальный мультипараметрический анализ на уровне одиночных клеток весьма затруднен и требует возможности анализа биомаркеров с использованием искусственного интеллекта для комплексного анализа всего вышеперечисленного пула профилей данных ("exploring such characteristics as potential biomarkers through single-cell omics and Artificial Intelligence (AI)"). Поэтому особые надежды возлагаются на платформы для раманомики, базирующиеся на конфокальных рамановских микроспектрометрах, для идентификации соответствующих "сигнатур" в объёме клеток - на клеточном либо органеллографическом уровне ("Another study used the Ramanomics platform, which coupled confocal Raman micro-spectrometry to a biomolecular component analysis algorithm to identify signatures") (Gayan et al., 2022).

Кросс-валидируемость данных раманомики.

Существенным преимуществом раманомики как позиционно-чувствительного омиксного подхода, работающего на уровне клеток и субклеточных структур, является сравнимость/совместимость его с масс-спектрометрией, которая является движущей силой прогресса в омиксных областях, в особенности – в позиционно-чувствительных "spatiotemporal omics" (Girolamo et al., 2013; Franceschi et al., 2013; Wolyniak et al., 2018; Sanders, Edwards, 2020; Zaikin, Borisov, 2021; Wang et al., 2022; Challen, Cramer, 2022; Pade et al., 2021). Данное утверждение верно и для спектрометрии ионной подвижности (Arthur et al., 2017; Causon et al., 2020; Bilbao et al., 2021; Delafield et al., 2022; Paglia et al., 2022) (см. также некоторые диссертации на тему омиксных приложений метода спектрометрии ионной подвижности (Donohoe, 2016; Lareau, 2016). Аналогично тому, как это постулируется в раманомике, для пользователя масс-спектрометрических омик наиболее инфрмативны не просто спектры, а пространственные распределения соединений, отраженные в форме 2D картирования с достаточным пространственным разрешением – "*mass spectrometric imaging-based multi-omics*" (Belizario et al., 2015; Quanico et al., 2017; Dewez et al., 2019; Chao, Zongwei, 2021; Smets et al., 2021; Zhao et al., 2022; Wang et al. 2023a; Zhao, Cai, 2023; Wang et al., 2023b; Phulara & Seneviratne, 2024). Также, как и в случае раманомики, предельным (не только в случае имэджинга, но и для всех масс-спектрометрических омик) разрешением считается анализ одиночных клеток – т.н. "*single cell omics*" или "*single cell multi-omics*" (DeLaney et al., 2018; Lu et al., 2023; Zhao et al., 2023; Zhang et al., 2023; Zhang, Qiao, 2024). При этом, в случае лазерных методов десорбции-ионизации, считается приемлемым сопряжение их с лазерной микродиссекцией клеток, как это имеет место и в рамановской спектроскопии и сопряженных с нею "омиках" (Quanico et al., 2017; Dewez et al., 2019). По сути, это есть, в методическом смысле, одно из ответвлений метода микропучковой пунктуры клетки, про которую мы недавно писали в обзоре (Orekhov, Gradov, 2023). И даже наиболее активно исследуемые объекты у раманомики (и, шире, спектраломики, включающей в себя иные вибрационно-спектрометрические омики) и масс-спектрометрических омик в достаточно существенной степени перекрываются (митохондрии (Wang et al., 2023b); нейротравмы и нейропатологии (Mallah et al., 2023); диагностика рака (Zhang et al., 2007; Nie et al., 2016; Pralea et al., 2020; Banerjee et al., 2023), включая область гепатоонкологии (Nie et al., 2016; Lawrence, 2023); и т.д.).

Поэтому, как минимум, в свете давних попыток инструментальной интеграции методов рамановской спектрометрии и MALDI-имэджинга (Bocklitz et al., 2013; Bocklitz et al.,2015; Ryabchykov et al., 2018), закончившихся возникновением подхода "RaMALDI" (который, по определению, представяет собой "simultaneous Raman and MALDI imaging"), следует полагать, что раманомика и масс-спектрометрические омики могут быть полностью интегрированы в один комплекс спектральных протоколов и комбинируемых инструментов, один из которых выдаёт на первой стадии данные неразрушающего аналитического контроля и идентификации субстанции, а второй, характеризующийся ионизацией, абляцией и десорбцией субстанции ("разрушающие"), на второй стадии эксперимента выдаёт полную качественную и количественную информацию о её составе. Этот подход применим как для живых, так и для биогенных и биокосных систем (Skottvoll, 2022; Luo et al., 2022).

Раманомика как комплементарный метод для микробиологического биотайпинга и редокс-патологии

Одним из приоритетных направлений обеспечения сопоставимости рамановского и МС картирования (в том числе – МС-имэджингового, например MALDI MS imaging) является микробиология. Рамановская микроскопия или рамановская микроспектрометрия часто используются в бактериологии, микробиологии (Huang, Spiers, 2006; Mosier-Boss, 2017; Lorenz et al., 2020; Hong et al., 2021; Jian, 2023; Burioni et al., 2024), в том числе в формате рамановской цитометрии, о которой было вскользь сообщено в предшествующем разделе (Jian, 2023). Результаты рамановских измерений в микробиологии хорошо соотносятся с результатами масс-имэджинга и омиксного картирования, а также – с предварительными данными идентификации микроорганизмов методами MALDI-биотайпинга (по MALDI биотайпингу см., например: Berrazeg et al., 2013; Somboro et al., 2014; Gekenidis et al., 2014; Pranada et al., 2016; Boyer et al., 2017; Houdelet, 2015; Antonios et al., 2022; Pena et al., 2022; по корреляционному имэджингу для MALDI-биотайпинга и миеробиологического мониторинга см. наши работы: Jablokow, Gradow, 2015a; Jablokow, Gradow, 2015b; Orekhov et al., 2016, Orekhov et al., 2023; Jablokow et al., 2017; Jablokow et al 2018; Orekhov, Gradov, 2022; Orekhov, Gradov, 2023a; Orekhov, Gradov, 2023b.

Ещё одним аспектом обеспечения сопоставимости рамановского и МС картирования, на наш взгляд, может стать окислительная модификация белков и анализ продуктов редокс-реакций в цитоплазме (в том числе, интерпретируемых в контексте насоновской теории/концепции "местной реакции протоплазмы" (Portugalov et al., 1964; Hadacek, Bachmann, 2015; Jaeken, 2017; Kosmachevskaya, Topunov, 2021; Bagatolli et al., 2021), впрочем, вполне безотносительно к его некорректным амембранистским воззрениям). Известно, что МС-, равно как и МС-имэджинг являются хорошими методами *in situ* анализа окислительной модификация белков и липидов (Person et al., 2003; Cornellison et al., 2011; Bykova et al., 2011; Murray, Van Eyk, 2012; Paulech et al., 2013; Bykova, Rampitsch, 2013; Butterfield et al., 2014; Bonham et al., 2014; Lennicke et al., 2016). Можно отметить, что методы MALDI MS + FRAP and FLIP (Jablokow, Gradow, 2015a, 2015b; Orekhov et al., 2016; Orekhov et al., 2023; Jablokow et al., 2017, Jablokow et al., 2018) также являются применимыми в целях цитофизиологического и цитопатологического редокс-анализа, так как, например, метод FRAP с генетически-кодируемыми редокс-сенсорными белками, такими как HyPer, давно и широко используется для картирования внутриклеточного распределения пероксида водорода и антиоксидантных градиентов в клетках и тканях (Belousov et al., 2006; Chudakov et al., 2010; Rhee et al., 2010; Samoylenko et al., 2013; Fernandez-Garcia, Olmos, 2014; Yang, 2014; Weller et al., 2014; Jones, Sies, 2015; Quintá et al., 2016; Delfosse et al., 2016; Bilan, Belousov, 2016; Rezende et al., 2018; Brilkina et al., 2018; Asada et al., 2018; Lyublinskaya, Antunes, 2019; Smolyarova et al., 2022). Из оптических методов известен ряд методов редокс-метрической микроскопии, фиксирующих повреждения внутри клетки или же их корреляты в атмосфере и окружающей среде, возникающие под действием тех же агентов (например, методы озонометрической микроскопии (Градов, 2012; Gradov, 2013). Раманомика же даёт возможность напрямую картировать происхождение активных форм кислорода, причём – неинвазивным и позиционно-чувствительным методом – с учётом компартментализации (Janků et al., 2019a, 2019b). В (Janků et al., 2019a) оптимистично резюмируется, что "“ramanomics” approach might provide an efficient tool of non-invasive quantitative profiling of cellular compartments and monitoring of molecular interactions" ("подход "раманомики" может обеспечить эффективный комплекс инструментов для неразрушающего-неинвазивного количественного профилирования клеточных компартментов и мониторирования молекулярных взаимодействий в них"). В работе (Janků et al., 2019b) указывается, что, кроме активных форм кислорода, можно анализировать на основе того же подхода компартментализацию окислов азота (NOx).

Раманомика как инструмент этиологического исследования в молекулярной онкологии

Примеры из вышецитированных работ (Janků et al., 2019a, 2019b) относятся к растительным клеткам, но, в действительности, ими данное направление ROS-метрии не ограничивается.

Так, например, возможно исследовать методами раманомики этиологию онкогенеза. Известно, что онкогенез нередко, в частности, связывают с:

– Накоплением активных форм кислорода (это давно известный подход, работы по которому встречаются как в старой литературе до 1990-го года (Fischer, 1987; Fischer et al., 1987), так и в работах 1990-х гг. (Standeven, Wetterhahn, 1991; Klein, Costa, 1991; Valavanidis, 1994; Emerit, 1994; Huang et al., 1994; Shi et al., 1998; Oliński, Jurgowiak, 1999), 2000-х гг. (Nishigori et al., 2004; Schulte-Hermann et al., 2006; Okada, 2007; Panayiotidis, 2008; Marquez-Quinones, 2007; Wang, 2009), 2010-х гг. (Ralph et al., 2010; Ziech et al., 2011, 2012; Grigorov, 2012; Tamura et al., 2013; Wu, Ni, 2015; Kruk, Aboul-Enein, 2017; Moldogazieva et al., 2018; Dupuy, 2018; Medeiros, 2018; Kovacic, Abadjian, 2018; Valavanidis, 2019; Jopkiewicz, 2019), 2020-х гг. (Gokulan et al., 2020; Vostrikova et al., 2020; Okazaki., 2022; Shimura, Ushiyama, 2024));

– Электрофилами (Chouchane, 1996; Miller, 1998; MacLeod et al., 2009; Smith et al., 2014; Olsen et al., 2018; Harach et al., 2019; Gobert et al., 2021; Lei et al., 2021; Danes et al., 2021) и шире понимавшимися на ранних этапах исследований в молекулярной и клеточной онкологии зарядовыми механизмами (Cavalieri, Calvin, 1972; Andrews et al., 1979; Shkarina et al., 1984; Kovacic et al., 1986);

– Зарядом мембраны и биоэнергетикой митохондрий (Tokuoka, Morioka, 1957; Beech, 1989a; Beech, 1989b, Beech, 1994; Marino et al., 1994; Ye et al., 2011; Friday et al., 2011; Yang, Brackenbury, 2013; Gogichadze et al., 2014; Lemeshko, 2015; Forrest, 2015; Lee et al., 2016; Li et al., 2020; Nnodim, Hauwa, 2020; Gąbka et al., 2021; Kuwahara et al., 2021; Sadri et al., 2022; Skates, 2022; Begum, Shen, 2023; Delisi et al., 2024).

В то же время, рамановская (микро)спектрометрия и раманомика, используемые в анализе онкогенеза по метаболическим нарушениям (которые, в частности, могут быть связаны с воздействием редокс-факторов) (Larion et al., 2018; Lawrence, 2023), могут быть использованы для коррелирования результатов редокс-повреждений или продуктов действия активных форм кислорода и онкогенеза.

3. Заключение

Из всего вышеизложенного вполне очевидно, что внедрение методов раманомики как в молекулярную медицину, так и в молекулярную биотехнологию является приоритетной задачей, следующей из всего развития последних десятилетий.

В следующей части данной работы, планируемой к выходу в 2025 году, мы рассмотрим практический аспект инструментального развития раманомики, начиная с молекулярно-оптических лазерных анализаторов (MOLE), которые мы пытались использовать для подобных задач много лет назад.

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Ретроспективный анализ схем рамономики: от количественной рамономики с использованием глубинных сверточных нейронных сетей для point-of-care-диагностики до молекулярно-оптических лазерных анализаторов. Часть 1 (Библиографический обзор)

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Аннотация. В данной обзорной работе производится ретроспективный анализ технологий рамономики и её методологических предшественников, направленный от современной количественной рамономики с использованием глубоких сверточных нейронных сетей (используемой для интраоперационной диагностики и point-of-care-диагностики) до Molecular Optical Laser Examiners (MOLE) 1970-х гг. Первая часть обзора, публикуемая в настоящем выпуске, рассматривает современные направления данного тренда, в то время как во второй части представляются достижения более раннего периода. В первой части внимание уделяется приложениям рамономики для диагностики супрамолекулярных патологий, механизмов апоптоза, парабиоза, онкогенеза, ряда редокс-патологий (а также эффектов воздействия активных форм кислорода на клетки и ткани), повреждений гематоэнцефалического барьера и нейротравм, затрагивающих цитоархитектонику мозга и, шире, архитектуру нейрональных коннектомов. Указывается ряд работ, позволяющих говорить о рамановском анализе в задачах спектральной сравнительно-патологической органеллографии цитоплазмы. Приводятся сведения об интегрируемости рамономики и методов масс-спектрометрического картирования или RaMALDI, в том числе – для задач MALDI-биотайпинга (как правило, используемого в клинической микробиологии).

Ключевые слова: рамономика, количественная рамономика, спектраломика, омиксный ультраструктурный анализ, MALDI-имэджинг, RaMALDI, синхронизированные MALDI-имэджинг и рамановская визуализация, безметочный времяразрешенный мониторинг на уровне одиночных клеток, интраоперационная диагностика, POC-диагностика, сверточные нейросети.

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